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THE PHILIPPINE
JOURNAL OF SCIENCE

VOL. 44 JANUARY–FEBRUARY, 1931 NOS. 1–2

EXPERIMENTAL STUDIES OF DENGUE[1]

By JAMES STEVENS SIMMONS, PRESIDENT
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Captain, Veterinary Corps, United States Army

THREE PLATES AND SIXTY-SEVEN TEXT FIGURES

INTRODUCTION

During the past thirty years the Medical Department of the United States Army has been actively interested not only in dengue but also in the similarly transmitted and more formidable disease, yellow fever. This interest, which was due, primarily, to the appalling mortality caused by yellow fever among our armed forces during the Spanish-American War and, later, to the extremely high morbidity rates for dengue and other infections among troops stationed in the Philippines, led to the formation of boards of medical officers, one in Cuba and another in the Philippine Islands, for the investigation of tropical diseases. The fundamental discoveries concerning the mosquito transmission of yellow fever, contributed by the board working in Cuba with Maj. Walter Reed in 1900, led to the almost complete eradication of that disease.

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[1] From the United States Army Medical Department Research Board, Bureau of Science, Manila, Philippine Islands. This article with an appendix, consisting of protocols of the mosquitoes and the human volunteers, will be reprinted as a Bureau of Science monograph.

The other board, appointed in 1900, for the investigation of tropical diseases as they exist in the Philippines, was dissolved in 1902, reformed in 1906, and after a period of inactivity from 1914 to 1922, was again reconvened and called the United States Army Medical Department Research Board. During this time succeeding members of the Philippine boards made valuable contributions to the knowledge of many of the important tropical diseases, including dengue. Ashburn and Craig, while working on this board in 1907, brought out certain fundamental information concerning the etiology, epidemiology, transmission, symptomatology, diagnosis, prophylaxis, and treatment of the disease. They reported the transmission of dengue experimentally to one of four volunteers with *Culex quinquefasciatus*, were the first to demonstrate the infective virus in the circulating blood of dengue patients, and were also first to establish the fact that the virus of dengue belongs to the group of so-called filterable viruses. In 1922 dengue was again made the subject of an extensive and important investigation by Siler, Hall, and Hitchens, while members of this board. These workers not only confirmed the fact that dengue is transmitted by *Aëdes ægypti*, but also determined the period of infectivity of dengue patients, the period required for development of the virus in *A. ægypti*, and the period during which infective *A. ægypti* are capable of transmitting dengue. They were unable to transmit dengue with *Culex quinquefasciatus*. Their comprehensive report on the history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity, and prevention of dengue furnishes a most important source of information concerning the disease. Schule, working on the board in 1928, continued these investigations and confirmed the work of Siler, Hall, and Hitchens regarding the period of time that must elapse between the infectious feeding and the transmission of virus to man by *A. ægypti*, and showed that under certain conditions this period may be as short as eight days.

In 1928 the authors of the present report selected dengue as a subject for further study. The more important of the problems considered had to do with (*a*) the epidemiology of dengue; (*b*) the nature of the virus, its identification, size, filterability, appearance, virulence, viability in vitro, and its possible growth in culture media; (*c*) the possibility of transmitting dengue to man by insects other than *A. ægypti;* (*d*) the possibility of transmission of dengue from infected to normal insects; (*e*) search for a lower animal susceptible to dengue which would be suitable for use, instead of man, in diagnostic and experimental work; (*f*) search for improved methods of diagnosis by studies of the leukocyte curves and serological reactions of patients; (*g*) further studies of the immunity to dengue of man and lower animals; (*h*) observations on the therapeutic value of immune sera; and (*i*) the investigation of a prophylactic vaccine.

The results, even of negative observations, are submitted in detail, as it is believed that the data brought out should not only add certain fundamental information to the present knowledge concerning dengue, but may also prove of assistance to those engaged in experimental investigations of both dengue and yellow fever.

EPIDEMIOLOGY OF DENGUE IN THE PHILIPPINE ISLANDS

Although dengue has existed in the Philippine Islands for many years, there is no reliable information concerning the incidence of the disease or its spread among the native and white civilian inhabitants. Factors that have made the collection of such data impossible are (*a*) the failure of a large proportion of the native population to seek competent medical attention, (*b*) the difficulty experienced even by well-trained physicians in differentiating mild or atypical dengue from other acute febrile conditions, and (*c*) the fact that dengue is not considered a reportable disease by the Philippine Health Service.

Therefore, the epidemiological studies of dengue made in the Philippines—including those made by Ashburn and Craig(1) in 1907; by Siler, Hall, and Hitchens(2) in 1924; and those of the present board—have been limited mainly to the disease as it occurs in military personnel, and have been based either on investigations made at Army posts or on official records in the office of the Surgeon General, United States Army, and of the Surgeon, Philippine Department. As Siler, Hall, and Hitchens have already published a comprehensive statistical analysis of dengue in the Philippines for the period 1902 to 1924, certain of their figures have been used along with additional official records for the period 1924 to 1929 in order to extend their studies to the present time.

Dengue in the United States Army.—Since the first American troops arrived in the Philippines, dengue, because of its high morbidity and the resultant loss of time from hospitalization, has been a source of considerable economic loss to the Army.

A study of the geographical distribution of dengue among American soldiers shows that its importance to the Army as a whole is due almost entirely to its high incidence in the Philippine Islands. The annual dengue admission rates per thousand for white enlisted men serving in different countries during 1928, as indicated in fig. 1, were as follows: (*a*) The entire Army, 6.84; (*b*) the Army in the United States, 0.02; (*c*) the Army in Hawaii, 0; the Army in Panama, 0.5; and (*d*) the

Army in the Philippine Islands, 177.06. Of the seven hundred sixty-two cases of dengue reported for white enlisted men in the entire United States Army during the year 1929, 97 per cent occurred in the Philippines. With minor variations these figures give a fairly accurate picture of the geographical distribution of the disease among American troops during previous years and indicate the great prevalence of dengue in the Philippine Islands.

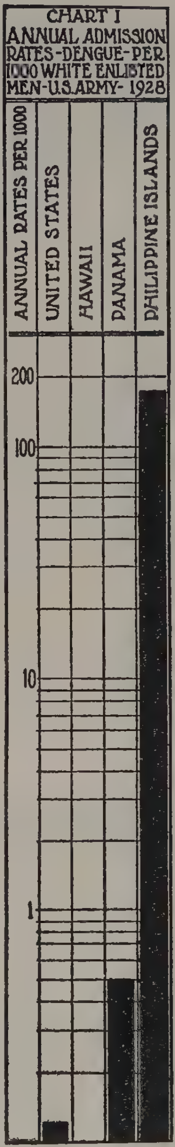

FIG. 1. Annual dengue admission rates per thousand white enlisted men in the United States Army, 1928.

Dengue in the Philippine Islands.—During the past twenty-seven years, as indicated in fig. 2, the annual admission rates from dengue have shown no sustained improvement, while during the same period the rates for all diseases considered together have decreased, indicating that with the exception of dengue there has been an improvement in the general health. In 1924 it was estimated by Siler, Hall, and Hitchens that during the twenty-five previous years dengue had been responsible for an average loss to the Army of 7,715 days per year; since 1924 the time lost has amounted to 1,978 days for 1925, 3,119 days for 1926, 5,450 days for 1927, and 5,226 days for 1928, an average loss per year of 3,943 days.

Distribution by Army posts.—The incidence of dengue differs strikingly at the various Army posts in the Philippines, as is indicated in fig. 3. Camp John Hay, which is situated in the mountains at an elevation of about 5,000 feet, has always been free from the disease, while the lowland posts in or near Manila have relatively high admission rates.

Camp John Hay is situated in the mountains of Luzon about 180 miles north of Manila at an elevation of approximately 5,000 feet. During 1928 the mean strength for white enlisted men was only 23, and for native soldiers 202. The temperature range for that year was as follows: Minimum, 12.5° C.; maximum, 29° C.; mean, 18° C.; and the annual rainfall was 178.24 inches. According to the

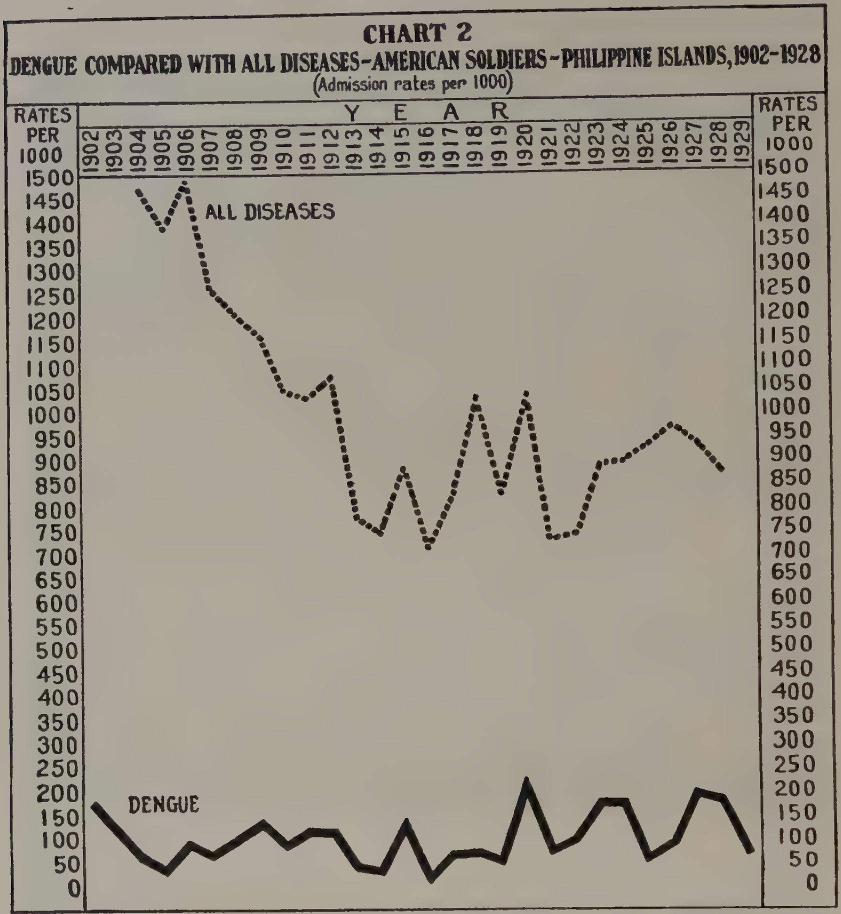

FIG. 2. Dengue compared with all other diseases contracted by American soldiers in the Philippine Islands, 1902–1928.

available information *Aëdes* mosquitoes have not been found there, although several species of *Anopheles* and *Culex* were collected in large numbers during April, 1929. It is claimed that dengue does not occur among the civilians living in the near-by City of Baguio; while the official records show that the disease does not occur either in the white or native soldiers of the military post. In this connection it is of interest to note that recently it has been shown experimentally by the authors that native Ifugaos living in this dengue-free post are extremely susceptible to dengue. Also that one native seen in April, 1928, had contracted the disease during a stay at Fort William

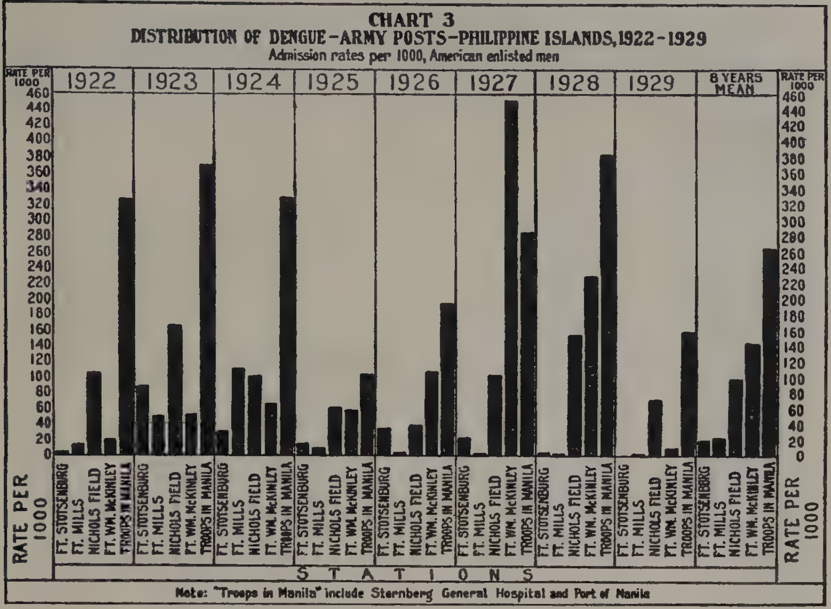

FIG. 3. Distribution of dengue among Army posts in the Philippine Islands, 1922–1929.

McKinley and had returned to Baguio during the second day of typical dengue. The high altitude and the resultant low temperature at Camp John Hay are probably responsible for the apparent freedom of that post from dengue.

Fort Stotsenburg, at an elevation of about 680 feet, is situated in Pampanga Province, 66 miles northwest of Manila, on a sloping sandy plain from which the reservation extends into mountainous country. The temperature range during 1927 was, average maximum, 32.5° C.; average minimum, 21.5° C.; with a daily variation of nearly 13.5° C. The average annual rainfall was 108 inches for the period 1916 to 1926, and the relative humidity for twelve months in 1927 was 91. There is no large city near this post, but several native barrios are located in the vicinity. The average yearly strength for American troops at Fort Stotsenburg has been as follows: 728 in 1922, 238 in 1923, 212 in 1924, 198 in 1925, 200 in 1926, 214 in 1927, 299 in 1928, and 190 in 1929.

The relatively low dengue admission rates for Fort Stotsenburg, indicated in fig. 4, have no doubt been due mainly to the isolation of the post and the active mosquito-control work which has been carried on there for several years.

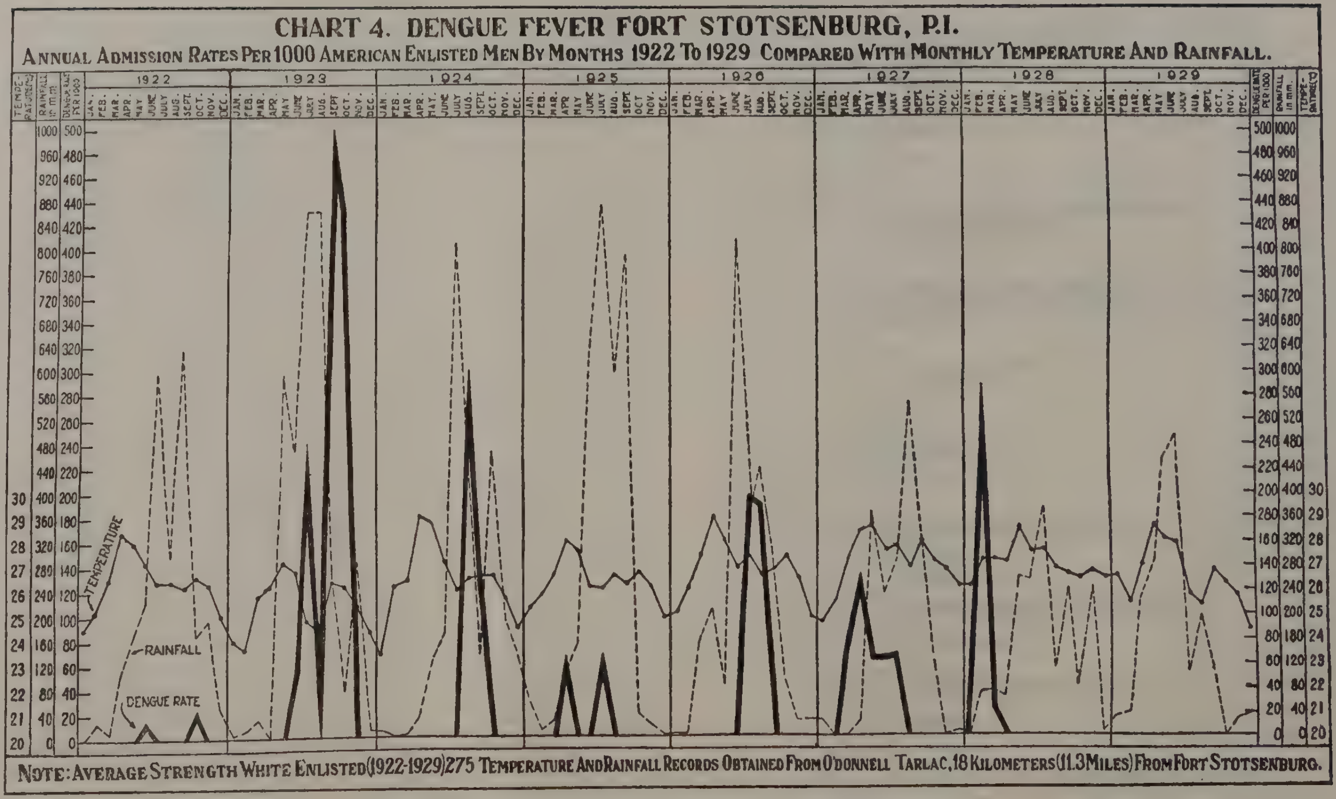

FIG. 4. Dengue fever at Fort Stotsenburg, Philippine Islands.

Fort Mills occupies a portion of a small island, Corregidor, situated in the mouth of Manila Bay about 48 kilometers from the City of Manila. The island rises abruptly from the sea to an elevation of about 500 feet. The temperature range for Corregidor during 1928 was as follows: Minimum, 19.5° C.; maximum, 36° C.; mean, 26° C.; and the total rainfall was 116.60 inches.

The dengue vectors, *Aëdes ægypti* and *Aëdes albopictus,* have been found breeding on the island, and were collected there by the authors during 1928. The white enlisted strength of this post was 2,352 in 1922, 1,057 in 1923, 1,363 in 1924, 1,406 in 1925, 1,372 in 1926, 1,264 in 1927, 1,464 in 1928, and 1,592 in 1929.

The incidence of dengue among troops at Fort Mills during the past seven years is indicated in fig. 5.

Nichols Field, which is located just outside Manila, has practically the same rainfall and temperature range as that of the city. The average strength for American troops was 296 in 1922, 398 in 1923, 447 in 1924, 472 in 1925, 473 in 1926, 454 in 1927, 448 in 1928, and 532 in 1929. As shown in fig. 6, dengue is not infrequent at this station.

Fort William McKinley, which is situated about 9 miles southeast of Manila at an elevation of not more than 125 feet, has practically the same average rainfall and temperature as Manila. While it is one of the largest stations in the Islands, the average strength for white American troops is relatively small as follows: 247 in 1922, 551 in 1923, 851 in 1924, 993 in 1925, 949 in 1926, 927 in 1927, 782 in 1928, and 409 in 1929. The dengue admission rates for this post are indicated in fig. 6.

Troops in the City of Manila.—The City of Manila is situated on Manila Bay in latitude 14° 35′ 31″ north and longitude 120° 58′ 08″ east, at sea level. During 1928 the total rainfall was 1,758.8 millimeters; and the maximum, mean, and minimum temperatures were, approximately, 36.7° C., 26.3° C., and 17.4° C., respectively. Troops stationed in the city include the personnel of the Sternberg General Hospital and the Post of Manila.

The dengue rates for all American troops stationed in Manila have been extremely high, as shown in figs. 3 and 6, in fact 69 per cent of all the dengue cases reported for the entire Army during 1928 occurred in this city. The importance of this source of dengue infection is further emphasized by the fact that if the adjacent stations, Fort William McKinley and Nichols Field, are

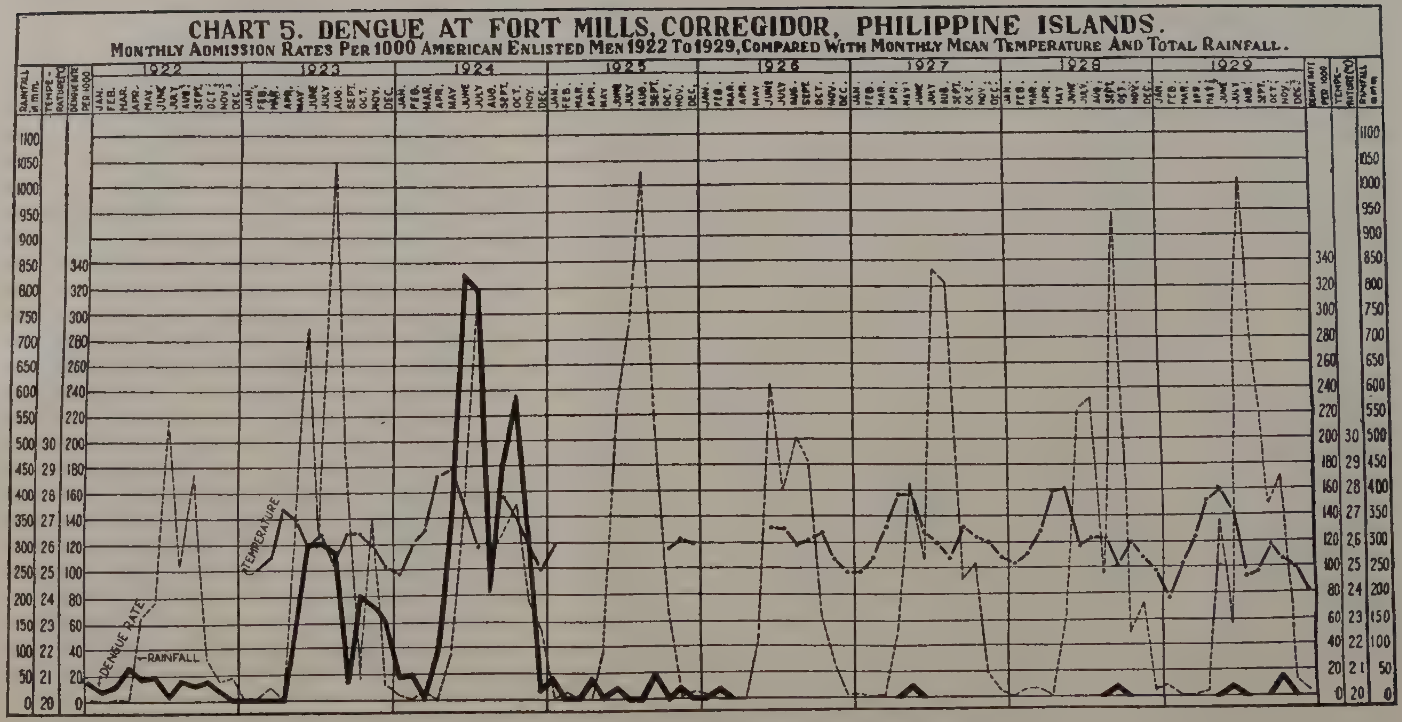

CHART 5. DENGUE AT FORT MILLS, CORREGIDOR, PHILIPPINE ISLANDS.
MONTHLY ADMISSION RATES PER 1000 AMERICAN ENLISTED MEN 1922 TO 1929, COMPARED WITH MONTHLY MEAN TEMPERATURE AND TOTAL RAINFALL.

FIG. 5. Dengue at Fort Mills, Corregidor, Philippine Islands.

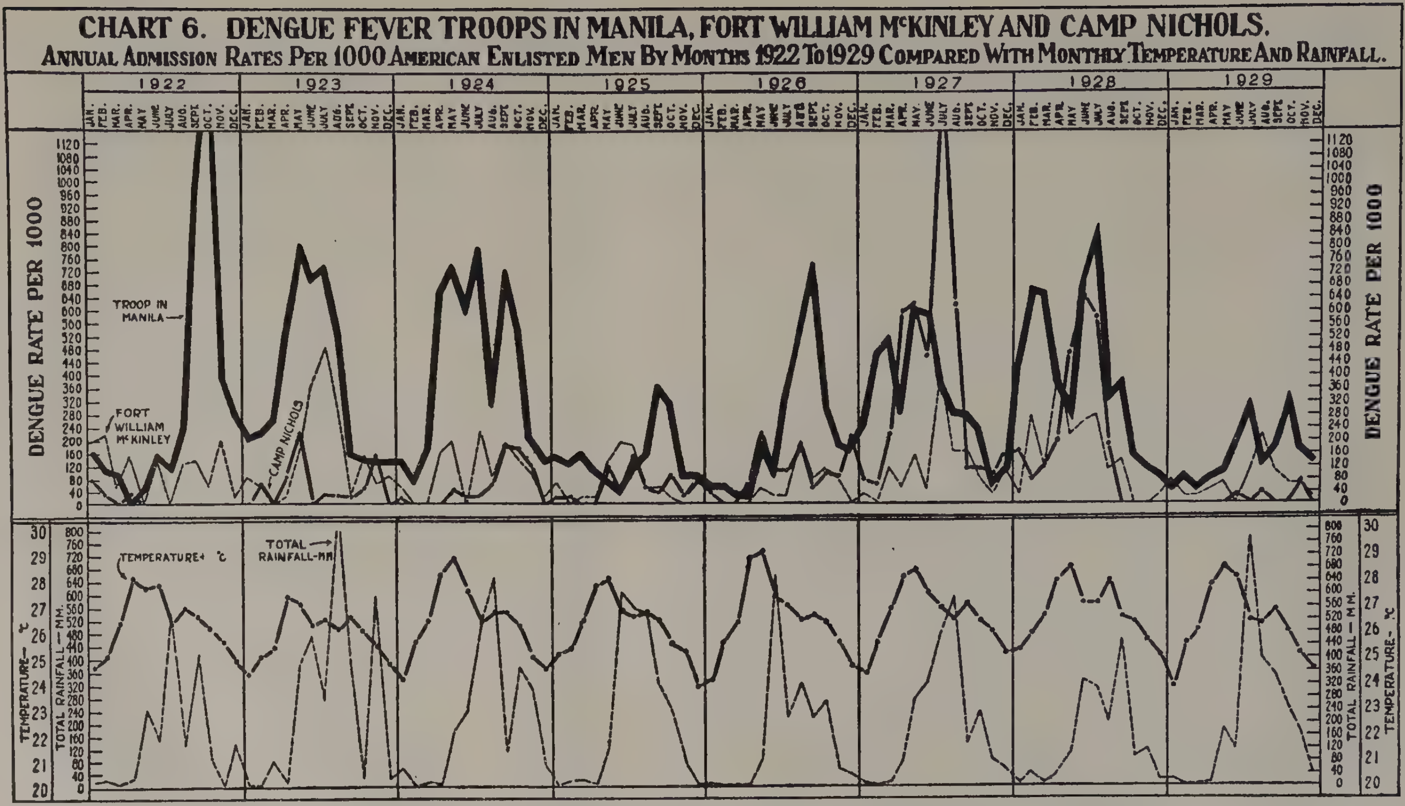

FIG. 6. Dengue fever among troops in Manila, at Fort William McKinley, and at Camp Nichols.

included, it may be stated that 98.2 per cent of the cases among troops in the Philippines, and 96.2 per cent of all cases reported among the 111,340 white troops in the entire United States Army during 1928 occurred among 2,406 American soldiers stationed in and near Manila.

DISCUSSION

From these observations it is apparent that the only important focus from which the American soldier is likely to contract dengue at present is in Manila or the immediately adjacent territory. The decided differences in the incidence of the disease among troops in the continental United States, Hawaii, Panama, and the Philippines cannot be explained entirely by climatic or other intrinsic differences in these various countries. While dengue may not be so common among the civil population of the United States or Hawaii as in the Philippines, still the disease is known to exist in both places, and epidemics have occurred in each of these countries as well as in Panama and in the Philippines. It is also known that an effective vector of dengue, *Aëdes ægypti,* is prevalent in each of these countries. Furthermore, Panama with an insignificant admission rate of 0.5 per thousand for 1928, and the Philippine Islands with an extremely high rate of 177.06, are both tropical countries. Thus, factors other than climate must be considered. While differences in the proportion of susceptible individuals from the dengue-free northern parts of the United States, assigned to duty in the different countries, and their length of stay no doubt influence the rates, it is believed that the most important factor is the degree of effectiveness with which troops are protected from dengue-infected mosquitoes in the various localities. In the Panama Canal Zone and surrounding areas the employment of every available scientific facility in mosquito prophylaxis has not only resulted in the disappearance of yellow fever and a marked decrease in malaria, but undoubtedly has also been responsible for the extremely low dengue rates among soldiers stationed there. Army posts both in the United States and Hawaii not only have the advantage of the mosquito control work of Army sanitarians, but also are to a large degree protected from dengue of civilian origin by the excellent sanitary conditions in neighboring towns and cities, where it is the custom for the people to live in screened houses. Screening alone undoubtedly plays an important part in reducing the proportion of mosquitoes infected and in keeping the dengue rates at a minimum in these localities. On the other hand, conditions in the Philippines are particularly favorable to the spread of dengue. Not only is the climate ideal for mosquito breeding, but also the lack of effective mosquito control among the civil population results in the development of enormous numbers of the vectors, *Aëdes ægypti* and *Aëdes albopictus;* while the custom, among both native and white civilians, of living in unscreened houses no doubt results in the exposure of a large proportion of dengue cases to innumerable mosquitoes. The latter factor alone would result in a much higher percentage of infected vectors than in the other countries mentioned. The introduction of new groups of nonimmune recruits into such highly infected areas of the Philippines at frequent intervals is inevitably followed by large numbers of dengue infections. However, in certain of the nine isolated Army posts in the Philippines active mosquito-control work and the screening of infected persons have resulted in a reduction of dengue rates. For instance, at Fort Stotsenburg which is removed from large towns or cities, the local mosquito-control work is also apparently effective in keeping down the rates for dengue. On the other hand, it is impossible by control work on the Army posts alone to protect troops stationed in Manila from dengue completely, so long as the soldiers are continually exposed to the great numbers of dengue-infected mosquitoes from other parts of this *Aëdes*-infested, unscreened city.

SEASONAL OCCURRENCE

Dengue occurs in seasonal waves in the Philippines, and as a rule these waves reach a high point some time between April and November. The monthly incidence of dengue in the Philippines between January, 1922, and December, 1929, is shown in fig. 7, along with the monthly temperature and rainfall for Manila. The mean monthly temperature curve for each year is lowest from November to April and reaches its height during the remaining months. The monthly rainfall, which varies from year to year, apparently plays an important part in determining the period of greatest mosquito prevalence, and consequently the time at which dengue is most prevalent. For example, by

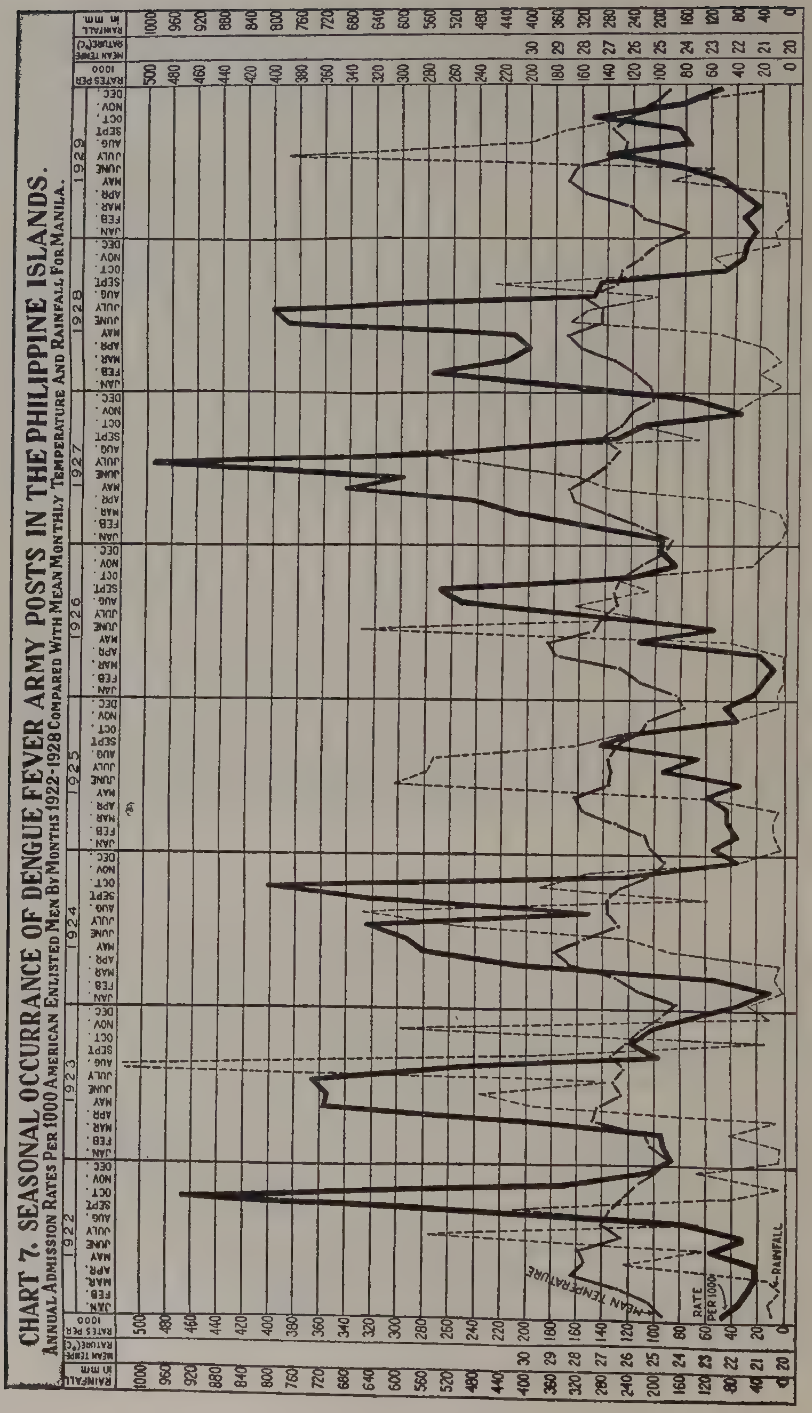

FIG. 7. Seasonal occurrence of dengue fever at Army posts in the Philippine Islands.

making daily observations throughout the period 1922 to 1924, Siler, Hall, and Hitchens determined that the late seasonal rise in dengue during 1922 was due to the peculiar daily distribution of rainfall during that year.

RACIAL DISTRIBUTION

It is unfortunate that more complete information is not available concerning the incidence of dengue among the native inhabitants of the Philippines. Such data would, no doubt, help to answer certain important questions concerning dengue immunity and might throw further light on the method of maintenance and dissemination of the virus in endemic localities. However, some information concerning dengue among adult male Filipinos is made available by the official records concerning Philippine Scout soldiers. As indicated in fig. 8 the annual dengue admission rates for Filipino troops since 1915 have been consistently much lower than the rates for American troops. It would appear that while dengue does occur among adult Filipinos, the native Scout soldiers as a group are not nearly so susceptible as American troops. This conclusion is substantiated by the fact that attempts to transmit dengue to Scout soldiers experimentally, both by Siler, Hall, and Hitchens and by the authors, have almost invariably failed. The former workers advanced the theory that the relative immunity of adult Filipinos is not a racial characteristic, but has probably been developed as a result of repeated exposure to infection since childhood. This supposition is concurred in, particularly since it has recently been proved that adult natives living in dengue-free mountain provinces of northern Luzon are just as susceptible to experimental infection with the disease as are white men.

Granting that native children are as a rule susceptible to dengue, it becomes apparent that the introduction of susceptible individuals from elsewhere is not necessary to the continued maintenance of the virus in an endemic area, but that the advent of children each year is sufficient to keep up the cycle of transfer indefinitely, even in isolated native communities. In addition, it is quite probable that mild unrecognizable infections may occur in many adults, as has been proved possible in monkeys, and that the virus can be transmitted from these apparently symptomless cases of dengue.

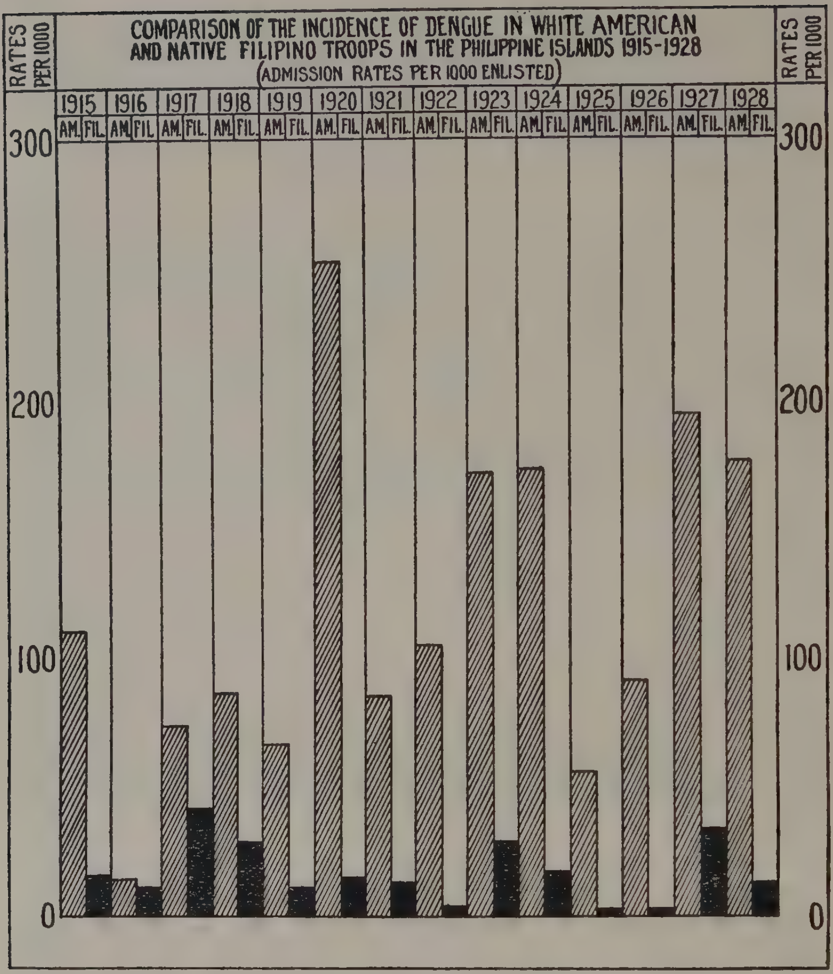

FIG. 8. Comparison of dengue incidence in white American and native Filipino troops in the Philippine Islands, 1915–1928.

ANIMAL AND INSECT RESERVOIRS OF DENGUE VIRUS

Another factor which demands consideration is the possibility of maintaining a reservoir of dengue virus by passage of the infection through animals, or by direct transfer of the virus from infected to normal mosquitoes. The authors have proved that at least a small proportion of adult monkeys living in endemic areas and a larger proportion from dengue-free localities may develop dengue without recognizable symptoms, and that from these monkeys the virus may be transmitted through mosquitoes to human beings. It has also been shown that under artificial conditions normal mosquitoes can be infected by the ingestion of suspensions of dengue-carrying mosquitoes, which suggests the possibility of such transfer of infection under natural conditions.

It, therefore, appears almost impossible to control the many unrecognizable sources of mosquito infection. While the protection of dengue-fever cases from mosquitoes by screening is a logical and advisable control measure, it seems quite probable that even if all recognized dengue cases were so protected, mosquito infection could not be prevented. Therefore, the most effective methods of control should be those that are directed mainly toward the reduction of the insect vectors.

INSECT VECTORS IN THE PHILIPPINES

At least two important vectors of dengue, *Aëdes ægypti* and *Aëdes albopictus*, are prevalent in the Philippine Islands. It has been proved that both of these mosquitoes are susceptible to infection with dengue virus and are effective in transmitting the virus to man. There is, of course, the possibility that other insects may also be concerned in the dissemination of the disease under natural conditions.

SUMMARY AND CONCLUSIONS

1. Dengue is of importance to the United States Army chiefly because of its high incidence among white American troops stationed in or near the mosquito-infested, endemic dengue area in and adjacent to the City of Manila, Philippine Islands.

2. Two important vectors, *Aëdes ægypti* and *Aëdes albopictus*, are known to be prevalent in the Philippines. It is of course possible that further investigation may show that other insects, including various species of mosquitoes and phlebotomus, are also capable of transmitting the disease.

3. Dengue shows a seasonal variation corresponding to changes in the numbers of mosquito vectors available, which factor is in turn influenced by variations in the rainfall and temperature.

4. In the Philippines the dengue admission rates for white American soldiers are much higher than for Philippine Scout soldiers.

5. The immunity of the adult Filipino soldier is probably due to repeated exposure to dengue during childhood.

6. The possibility is mentioned that dengue virus may be maintained in an endemic area by transfer through (*a*) susceptible native children, (*b*) incompletely immune native adults, (*c*) susceptible newcomers from dengue-free localities, (*d*) susceptible young animals or incompletely immune adult animals, and possibly (*e*) by direct transfer from infected to normal insect vectors.

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TRANSMISSION OF DENGUE BY AËDES ÆGYPTI

The great importance of *Aëdes ægypti* as a dengue vector was fully demonstrated by the experiments of Bancroft,(1) Cleland, Bradley, and McDonald,(2) and Siler, Hall, and Hitchens;(3) and information concerning the mechanism of its transmission of dengue was furnished by the extensive investigations of the last-mentioned group of workers. During the present studies, including a large number of experiments in which were employed one hundred thirty-one human volunteers, forty-three monkeys, many smaller laboratory animals, and two hundred twenty-six lots of mosquitoes, mostly *A. ægypti* (see fig. 9), considerable information has accumulated bearing on the mechanism of dengue transmission by this mosquito. These observations are of interest chiefly because they substantiate the conclusions of Siler, Hall, and Hitchens(3) concerning *A. ægypti* and prove that the findings of these authors may be safely applied to general experimental work with this mosquito. So far as possible throughout the present studies the normal mosquitoes were fed on infective blood during the first or the second day of dengue; and were used for the transmission of dengue only after twelve or more days had elapsed. Laboratory-bred *A. ægypti* descended from the stock used by Siler, Hall, and Hitchens in 1924 and a single strain of dengue virus were used in all of the experiments.

PERIOD DURING WHICH THE BLOOD OF DENGUE PATIENTS IS INFECTIVE TO AËDES ÆGYPTI

As the primary consideration in our work was to infect the mosquitoes, an effort was made in all cases to feed the normal *Aëdes ægypti* on dengue patients as early as possible after the onset of the disease. Of thirty-four lots of *A. ægypti,* Table 1, which took blood from cases of experimental dengue fever in which the onset was definitely known, twenty-nine ingested the infective blood during the first twenty-four hours, four in the second day, and one lot on the third day. The only failure occurred with *A. ægypti* of lot 11, which took blood from an American volunteer (case A.V. 5–M), who for three days previously

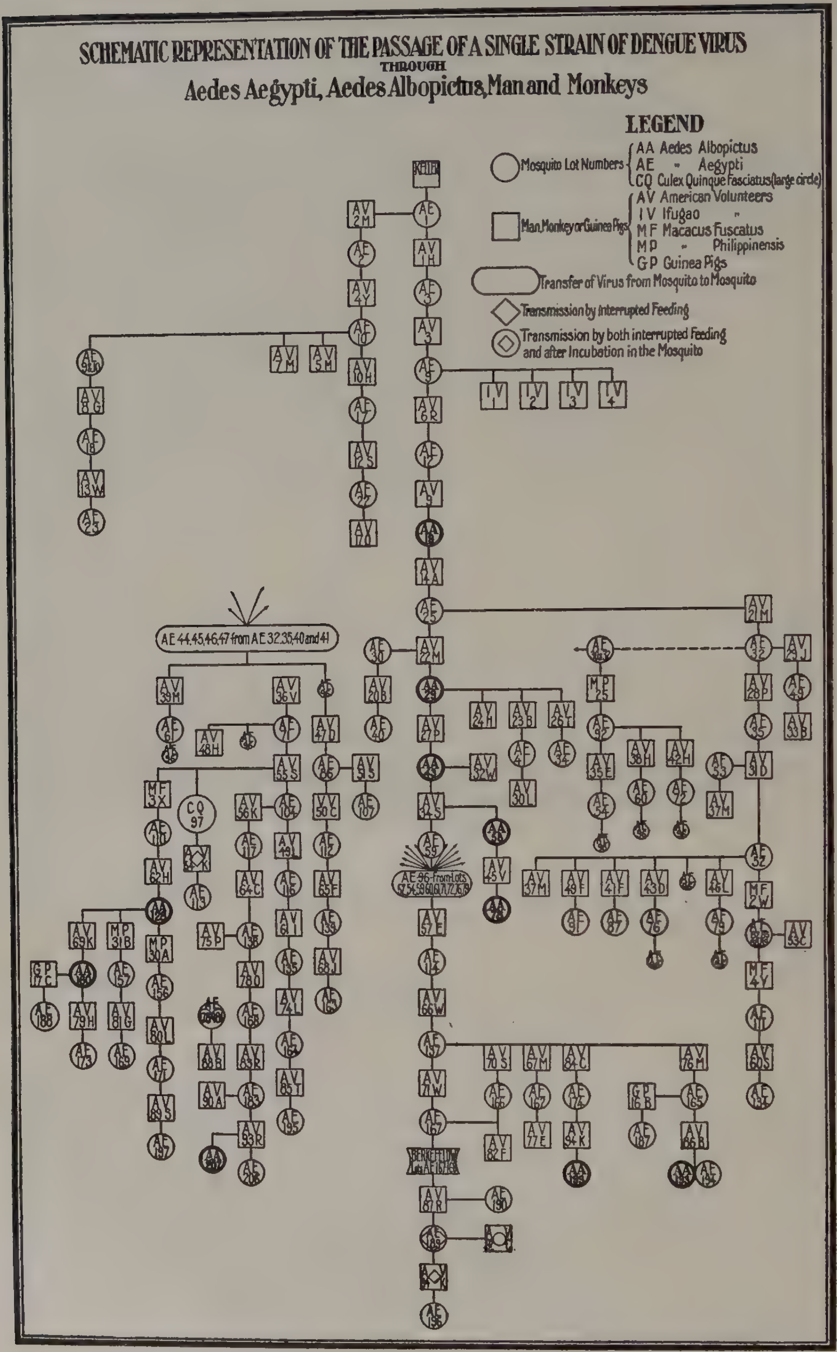
SCHEMATIC REPRESENTATION OF THE PASSAGE OF A SINGLE STRAIN OF DENGUE VIRUS
THROUGH
Aedes Aegypti, Aedes Albopictus, Man and Monkeys
LEGEND
Mosquito Lot Numbers
AA Aedes Albopictus
AE " Aegypti
CQ Culex Quinque Fasciatus (large circle)
Man, Monkey or Guinea Pigs
AV American Volunteers
IV Ifugao "
MF Macacus Fuscatus
MP " Philippinensis
GP Guinea Pigs
Transfer of Virus from Mosquito to Mosquito
Transmission by interrupted Feeding
Transmission by both interrupted Feeding and after Incubation in the Mosquito
AE 44,45,46,47 from AE 32,35,40 and 41
AE 96 From Lots
BERKEFELD

FIG. 9. Schematic representation of the passage of a single strain of dengue virus through various hosts.

Table 1.—*Interval between the feeding to infect Aëdes ægypti and subsequent use in dengue-transmission experiments.*

Interval in days.	Lot Nos. [a]	Total volunteers used.	Dengue fever.	Interval in days.	Lot Nos. [a]	Total volunteers used.	Dengue fever.
1	100	1	No.	27	112	1	Yes.
3	25	1	No.	29	173	1	Yes.
5	25, 69 [b]	2	No.	30	71, 174	2	Yes.
7–9	178, [b] 181 [b]	1	No.	31	32	1	Yes.
12	17, 32, 96	3	Yes.	32	52	3	Yes.
13	11	2	No.	37	19, 52, 171	3	Yes.
	30	1	Yes.	40	3, 10, 198	3	Yes.
14	53, 69, [b] 86, 116	4	Yes.	41	2, 52, 139	3	Yes.
15	25, 104, 162	5	Yes.	42	137	1	Yes.
15–17	178, [b] 181 [b]	1	Yes.	45	9, 135, 195	3	Yes.
16	11	1	No.	46	165	1	Yes.
	1, 18	3	Yes.	47	137	1	Yes.
17	117	1	Yes.	48	138	1	Yes.
18	49	1	Yes.	52	10, 137, 164	4	Yes.
19	167, 168	2	Yes.	54	190	1	Yes.
20	41	1	Yes.	55	9	3	Yes.
22	12, 35	2	Yes.	57	138	1	Yes.
24	114	1	Yes.	57–59	9 and 10 combined	1	Yes.
25	71, 86	2	Yes.	60	9	1	Yes.
25–29	167, 168	1	Yes.	70	137	1	Yes.

[a] All lots except lot 11 were proved infected in twelve or more days following the infective feeding.

[b] Lots 69, 96, 178, and 181 were infected by feeding on suspensions of dengue-infected mosquitoes, while all other lots were infected by feeding on experimental cases of dengue fever within forty-eight hours after the onset, excepting lot 11; this lot was fed during the third day after the first appearance of symptoms.

had shown marked prodromal symptoms. While the day of onset could not be definitely determined in this case, it is believed that the mosquitoes were fed on the third day, and that this accounted for the fact that they remained uninfected.

While exact experimental information is not available to show that each individual mosquito in the lots fed during the first two days of the dengue was infected, the fact that the disease was transmitted by all of these lots regardless of the numbers of mosquitoes used indicates that the infective rate must have been high.

TIME REQUIRED BEFORE AËDES ÆGYPTI, FED ON MATERIAL CONTAINING DENGUE VIRUS, WERE ABLE TO TRANSMIT THE DISEASE

It was concluded by Siler, Hall, and Hitchens (3) that the virus of dengue "must remain in the female *Aëdes* for a period of more than ten days—and that the evidence indicates that even on the eleventh day after infection of *Aëdes* the virus may not have reached such a state that the bite of the mosquito is constantly infective." While successful dengue transmission has occasionally been obtained following a shorter period of virus "development," as, for example, in the experiment reported by Schule(4) in which the period was only eight days, an interval of at least eleven days was usually allowed in the present work. Exceptions to this rule were a few lots used as controls in other experiments. For example, lot 25 fed on the third and fifth days, lot 69 fed on the fifth day, lot 178 fed on the ninth day, and lot 181 fed on the seventh day after the ingestion of dengue virus failed to transmit the infection to susceptible volunteers (Table 1). However, all of these lots were proved to be infective when used after longer periods of time had elapsed. In sixty-three successful transmission experiments with *A. ægypti* the intervals between the ingestion of infective blood and the subsequent test of the mosquitoes for infectivity were from twelve to seventy days (Table 1). Three of these lots (17, 32, and 96) were tested on the twelfth day after the meal of infective blood.

DURATION OF THE PERIOD OF INFECTIVITY IN AËDES ÆGYPTI

Siler, Hall, and Hitchens,(3) who reported the transmission of dengue by mosquitoes that had been infected sixty-two, sixty-six, and seventy-five days previously, made the following statement: "We believe the evidence justifies the statement that after the female *A. ægypti* has become infective she is capable of transmitting the virus of dengue throughout the remainder of her natural life." Blanc and Caminopetros(5) have been able to keep *A. ægypti* alive for seven months and have transmitted dengue with them one hundred seventy-four days after infection. Their observations concerning the influence of low temperatures on the period of infectivity are of particular interest as they may answer some of the obscure questions concerning the epidemiology of the disease. According to these workers infective *A. ægypti* may lose their ability to transmit dengue virus if kept at temperatures below 18° C.; and they state that mosquitoes kept in Athens remained infective up to January 13, but that the same mosquitoes were not infective during the cold months of February and March. In this connection it is interesting to note the record of our lot 190. Immediately after an infective blood feeding a portion of this lot was kept in the refrigerator at 18° C. for thirty days and subsequently at room temperature (26.3° C.) for twenty-four days, after which the mosquitoes were able to transmit dengue.

In the present work all except a portion of one lot were used in Manila where the maximum, mean, and minimum temperatures for the period were 36.7° C., 26.3° C., and 17.4° C., respectively. The lot that was used outside of Manila was taken to Baguio, Mountain Province, in April, 1929, and used the following day in transmitting dengue to each of four Igorot volunteers.

The longest periods of infectivity observed in the various lots happened to be sixty days with lot 9, and seventy days with lot 137 (see Table 1). Either because of death of the mosquitoes or for other reasons no lots were tested after longer periods of time.

Thus it may be stated that in the present work in Manila *A. ægypti* fed on infective dengue virus were subsequently able, in all instances, to transmit dengue between the twelfth and seventieth days.

NUMBERS OF INDIVIDUAL MOSQUITOES USED IN THE TRANSMISSION OF DENGUE

The numbers of potentially infected *Aëdes ægypti* used in successful transmission experiments varied from five to one hundred fifty mosquitoes for each volunteer. As shown in Table 2, over 8 per cent of the infections followed the feeding of from one hundred to one hundred fifty mosquitoes; 21.66 per cent were caused by fifty to one hundred mosquitoes; 43.33 per cent by ten to fifty mosquitoes; and 26.66 per cent of the cases were due to the bites of from five to ten mosquitoes. As no negative results were obtained except in the case of individuals later proved to be immune, it is apparent that small numbers of infective mosquitoes may be used for dengue transmission with the same certainty of positive results as if larger groups were employed. As sixteen, or 26 per cent, of the cases were infected by different lots of *A. ægypti,* all of which were made up of between five and ten individual mosquitoes, and as there were no failures after using such small numbers, it is apparent that at least one out of ten mosquitoes in these particular lots were infective. However, it is believed that such an estimate is far from exact; and since infections have been produced with one, two, or three individual *A. albopictus,*(6) it seems quite probable that of all normal *A. ægypti* which take blood from dengue cases during the infective period practically 100 per cent become infective, and that any individual mosquito in the group may become able to transmit dengue.

TABLE 2.—*Numbers of Aëdes ægypti used in the transmission of dengue to man and monkeys.*

Infective *A. aegypti:* lot Nos.	Number of mosquitoes fed.	Volunteers or monkeys infected with dengue.		Per cent of total cases.
		Number of cases.	Per cent.	
9	5	1	100	26.66
9, 52, 137	6	4	100	
9, 137	7	2	100	
32, 52, 71 (9 and 10)	8	4	100	
30, 52	9	2	100	
52, 86, 112	10	3	100	
Total for group	5– 10	16	100	
1, 12, 17, 19, 25, 41, 86, 100, 168, 171	10– 20	12	100	43.33
104, 137, 138, 165 (44, 45, 46, and 47) (9 and 10)	20– 30	7	100	
96, 100	30– 40	2	100	
9, 53, 135 (44, 45, 46, and 47) (178 and 181)	40– 50	5	100	
Total for group	10– 50	26	100	
2, 3, 49, 117, 69	50– 60	5	100	21.66
71, 137, 138	60– 70	3	100	
189	70– 80	1	100	
18, 35, 137, 162	80– 90	4	100	
Total for group	50–100	13	100	
10, 114, 139, 167	100–150	5	100	8.33
Total infections	5–150	60	100	100.00

SUMMARY AND CONCLUSIONS

1. The results of these experiments indicate that all lots of *Aëdes ægypti* that fed on blood from experimental cases of dengue during the first forty-eight hours of the disease became infected; while one lot which probably took blood during the third day of the disease failed to transmit the disease.

2. In a few experiments in which *A. ægypti,* one, three, five, seven, and nine days after they had ingested virus, were allowed to feed on volunteers the mosquitoes failed to transmit the infection to susceptible volunteers. However, all transmission experiments in which the intervals were from twelve to seventy days were successful.

3. No evidence was obtained to indicate that the infectivity of the mosquitoes was ever lost at the temperatures that prevail in Manila. Lots infected sixty and seventy days previously transmitted dengue as did mosquitoes infected for shorter periods.

4. It appears that when a group of normal *A. ægypti* ingests dengue virus a large proportion of the individual mosquitoes become infected. Dengue was transmitted by small lots composed of five to ten mosquitoes just as consistently as by large lots made up of one hundred to one hundred fifty mosquitoes.

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TRANSMISSION OF DENGUE BY AËDES ALBOPICTUS SKUSE

While *Aëdes ægypti* is the only species that has been generally accepted as a vector of dengue during the past few years, several other mosquitoes, including species of *Aëdes* and *Culex*, have been suspected either on epidemiological or experimental grounds. The recent discovery by Bauer(1) that several species of *Aëdes* are capable of transmitting yellow fever suggested the advisability of investigating the prevalence in the Philippines of species of *Aëdes*, other than *A. ægypti*, in order to determine the possibility of their acting as dengue vectors.

Collections of larvæ and adults from Manila and adjacent localities have yielded only two species of *Aëdes;* namely, *Aëdes ægypti* and *A. albopictus*. Attention of the board was directed to *A. albopictus* in February, 1929, during the examination of the first group of adult mosquitoes submitted for examination, from Corregidor Island. The results obtained in collections of larvæ made in representative parts of Manila during April, May, June, July, and August, 1929, indicated, as shown in Table 3, that both *A. ægypti* and *A. albopictus* are common mosquitoes in this locality. Hence, it was concluded that if *A. albopictus* were able to transmit dengue, its importance might be about the same as that of *A. ægypti*.

TABLE 3.—*Relative prevalence of Aëdes albopictus Skuse and A. ægypti in Manila, as indicated by collections of larvæ between April and September, 1929.*

Month.	Number of samples collected.	Samples containing *A. albopictus.*	Predominant species in samples.	Dengue cases admitted to Sternberg General Hospital, Manila.
		Per cent.		
April	1	100	*A. ægypti*	18
May	3	100	do	16
June	4	75	*A. albopictus*	38
July	16	100	Neither	33
August	104	43	*A. ægypti*	11

While systematic collections of *Aëdes* larvæ have not been made since August, 1929, adult *A. albopictus* have been identified in various localities. Specimens have been caught at Fort William McKinley by Maj. John Dibble, M. C., United States Army; it has been found as commonly as *A. ægypti* around Novaliches, Rizal Province, Luzon, by Dr. C. Manalang, chief of the malaria-control division of the Philippine Health Service; and specimens have been taken in Manila by Dr. Paul F. Russel, field director, International Health Division, Rockefeller Foundation. *Aëdes albopictus* has also been seen in the City of Manila during every month of the year by the authors, and during February and March, 1930, this mosquito was found to be the predominant species around the Manila residence of one of the authors.

Not only is *A. albopictus* a common local mosquito at present, but there is sufficient evidence to indicate that it has been prevalent in the Philippine Islands during the past. For example, Ludlow(2) in 1903 described this mosquito under the name of *Stegomyia scutellaris samarensis;* and in 1908(3) remarked that "in places where *Stegomyia calopis* was infrequent its place was largely taken by *Stegomyia scutellaris.*" Banks(4) in 1906 reported that he had failed to collect *S. scutellaris* Walker, but that *S. scutellaris samarensis* Ludlow "seemed a widespread mosquito in the Philippines." He noted that *S. scutellaris* had been found in the following locations: Pangasinan, Camp Gregg, Bayambang (Col. W. P. Chamberlain, M. C., U. S. Army), Samar, Leyte, Mindoro, Iloilo, Occidental Negros, Bago (Banks), Manila, Fort William McKinley (Col. Charles F. Craig, M. C., U. S. Army). As the names *S. scutellaris* Walker and *S. scutellaris samarensis* Ludlow are accepted synonyms for *A. albopictus* Skuse, it is apparent, from the information presented, that this common Oriental mosquito is widely distributed throughout the Philippine Archipelago. That *A. albopictus* is also prevalent throughout the Orient is indicated by the following observation made by Theobald(5) in 1901 concerning *Stegomyia scutellaris* Walker (*A. albopictus*):

> *Habitat.*—Singapore (Rafflesian Museum) (4. 9. 1899); Hong Kong (Ford) (27. 9. 1899); Selangor (A. L. Butler) (28. 10. 1899); Upper Burma (Watson); North Borneo; Mauritius (Sir Charles Bruce) (22. 11. 1899); Tamsui, Formosa (Mackay) (2. 8. 1899); Fiji (Black) (30. 12. 1899); Japan (Wood); Celebes (Walker); Ceylon (Bartholomew) (12. 12. 1899); Madras and Naini Tal, India (Giles and Cornwall); Siam

(Skeate); Amboina (Doleschall); Sombalpur, C. P., India (D. O'C. Murphy) (99); Foo-Chow, China (Rennie) (9. 8. 1900) (84).

Time of capture.—Singapore, July (July 27, 1899); Ceylon, November; Upper Burma (March).

Observations.—This is a very common mosquito, with a wide distribution in Asia. It is a common species in the Straits Settlements, being the second commonest mosquito in Selangor (A. L. Butler). Ford records its larvae as being abundant "in standing water near houses 500 feet above the sea." It is a great nuisance at Calcutta (Skuse). Skeate also evidently took it in abundance in Siam, for numbers are in the collection sent me by Dr. Sharpe.

Barraud,(6) in 1922, also called attention to the prevalence of *A. albopictus* and noted that there were specimens in the central Malaria Bureau collection from the following places:

North-West Frontier:—Kohat (Sinton).

Punjab:—Simla and Delhi (Christophers). Kasuali (Christophers and Sinton). Amritsar (Barraud).

Bombay:—Islands in the harbour, and Trombay (Barraud).

Bombay Deccan:—Belgaum and Nagargali (Barraud).

Malabar Coast and Nilgiri Hills (Khazan Chaud).

Madras Carnatic:—Madras (Patton). Salem (Christophers).

Bihar; Cuttack (S. Sundar Rao). Burma:—Rangoon (Christophers).

The following additional localities are taken from Theobald (Mon. Cul.):

Calcutta (Annandale); Upper Burma (Watson); Madras and Naini Tal (Giles and Cornwall); Sombalpur, Cen. Prov. (D. O'C. Murphy); Kanara district (E. H. Aitken); Sylhet, Assam (Major Hall); Lushai Hills, Assam (E. C. Macleod); Manipur (C. A. Gourlay); Katihar and Purneah district; N. Bengal (C. A. Paiva); Ceylon (Green).

It is of interest to note that *Stegomyia scutellaris* (*A. albopictus*) has been previously suspected as a possible vector of dengue on epidemiological grounds; and that during the 1915 dengue epidemic in Formosa, it was used in dengue-transmission experiments by Koizumi, Yamaguchi, and Tonomura.(7) However, as was pointed out by these workers, their results were inconclusive because the human volunteers used had not been previously protected from accidental infections, and while the mosquito was incriminated, it was not proved to be capable of transmitting dengue.

EXPERIMENTAL

MATERIALS AND METHODS

(*a*) *Source of dengue virus.*—The strain of virus used in these experiments was obtained originally December 15, 1928, from a naturally acquired case of dengue (Keller) and had been passed alternately through various lots of mosquitoes and either man or monkey. As indicated in

Chart 1, the virus transferred from A.V.[2] 22–M to *A. albopictus* lot 29 and on to lot 43 and lot 58 had already passed through six different lots of *A. ægypti* and six white American volunteers. As shown in Chart 2, the virus used in infecting *A. albopictus* lots 128 and 160 had already passed through ten lots of *A. ægypti*, nine American volunteers, and one Japanese monkey, *Macacus fuscatus* 3 (X). The virus used in infecting *A. albopictus* lot 183 had passed through thirteen lots of *A. ægypti*, and twelve American volunteers as indicated in Chart 3.

(*b*) *Source of Aëdes albopictus.*—The mosquitoes selected for use in the first five transmission experiments and known as lot 29 had emerged both from larvæ collected in Manila and from others bred in the laboratory. All other lots (43, 58, 128, 160, and 183) were descended from laboratory-bred mosquitoes. The specific identity of each mosquito used, either for breeding or in the transmission experiments, was verified immediately after emergence, before and after being placed in the breeding cages, before and after taking an infective blood feeding from a dengue patient, and before and after the tests on volunteers. Each mosquito used conformed to the description of *A. albopictus* given by Theobald (5) and Barraud, (6) (see Appendix), and the identity of the species was further confirmed by the Bureau of Entomology, Washington, D. C.

(*c*) *Human volunteers and monkeys used in the transmission experiments.*—The twelve white American soldier volunteers[3] used in these experiments were selected and handled as in other transmission experiments carried out during this investigation. After admission to the special dengue ward at Sternberg General Hospital, Manila, they were isolated in screened cubicles within the screened ward, for eight or nine days before the beginning of the tests; or, if volunteers who were already in the ward were used, this period of time was allowed to elapse between previous negative experiments, and the date *A. albopictus* was tested for infectivity.

The two Philippine monkeys (*Macacus philippinensis*) used with *A. albopictus* were caught in a dengue-free region at an elevation of about 4,000 feet in the mountains of northern Luzon, transported in screened boxes to Manila, and kept in a screened cage until discarded. They had not been used previously for experimental work and had probably never been bitten previously by dengue-infected *Aëdes* mosquitoes. These animals were observed in screened cages for periods of one or more weeks before infected *A. albopictus* were allowed to feed on them.

Technic.—In the transmission experiments with *A. albopictus* the procedure was in general similar to that used in the work with *A. ægypti*. The female mosquitoes were identified, placed in a "feeding cage," and given an opportunity to take blood from an experimental case of dengue during the first or second day of the disease. After the mosquitoes had fed, they were held for twelve or more days before they were tested for infectivity. After the potentially infective biting, the volunteer or animal was examined daily for evidence of infection; and in all instances records were made of the symptoms, temperature, and leukocyte counts.

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[2] American volunteer.

[3] Hereafter referred to specifically as "A.V." with a number and a letter.

Dengue was recognized either by the appearance of typical symptoms including a definite fever with leukopenia and an increase in the immature granulocytes, or by passage of the virus through other mosquitoes, and again to man, causing subsequent infection. As shown in the following experiments, the six different lots of *A. albopictus* used produced dengue in two monkeys (*Macacus philippinensis*) and in twelve volunteers.

Experiments with Aëdes albopictus, lot 29.—July 16, 1929. One hundred recently emerged female mosquitoes were given an opportunity to feed on the infective blood of an experimental case of dengue (A.V. 22–M) during the first twelve hours of the disease. Eighty of these mosquitoes took full feedings of blood within a short time, were designated lot 29, and were used in the following experiments.

Experiment 1.—July 29, 1929. Twelve *A. albopictus* of lot 29, fed on a volunteer (A.V. 23–B) who had been used previously for negative experiments July 1, 11, and 20, and had been observed for nine days prior to this test. After an incubation period of seven days dengue fever developed on the morning of August 5, 1929. The symptoms were typical, including fever, both initial and terminal rash, weakness, pains and aches in the eyes, knees, and elbows, and leukopenia. The diagnosis was further confirmed by the fact that normal *A. ægypti* fed during the first day of the disease and, designated lot 41, later transmitted the disease to another volunteer (A.V. 30–L). Comment: The feeding of twelve infected *A. albopictus* of lot 29 on a human volunteer was followed in seven days by typical dengue fever.

Experiment 2.—July 29, 1929. Eight *A. albopictus* of lot 29 fed on a volunteer (A.V. 26–T), who had previously been used for negative experiments July 11 and 21, and had been observed for eight days, prior to this experiment. After an incubation period of six days, dengue fever developed in the afternoon of August 4, 1929. The symptoms, which were typical of dengue, included fever, generalized rash, and leukopenia. Comment: The feeding of eight *A. albopictus* of lot 29 on this volunteer was followed in six days by typical dengue fever.

Experiment 3.—August 6, 1929. Three *A. albopictus* of lot 29 fed on a volunteer (A.V. 25–D) who had been used previously for negative experiments July 11, 21, and 29, 1929, and had been observed for eight days prior to this test. After an incubation period of six and one-fourth days dengue fever developed during the afternoon of August 12, 1929, with the typical symptoms of a mild case, without any initial rash. The infection lasted five days, ending with a terminal rash August 17, 1929. Comment: The feeding of three *A. albopictus*, lot 29, was followed in six and one-fourth days with typical dengue fever.

Experiment 4.—August 6, 1929. One infected *A. albopictus* of lot 29 fed on a volunteer (A.V. 27–P) who had been used previously for negative experiments July 11, 21, and 29, 1929, and had been observed for eight days prior to this test. After an incubation period of five days dengue fever developed in the afternoon of August 11, 1929, with fever, anorexia, vomiting, dizziness, headache, pains in the muscles and eyes, and typical leukopenia. On the second day a rash appeared; general glandular enlargement was noted later in the disease. The diagnosis was further confirmed by the fact that normal *A. albopictus* fed during the first day of the disease, and designated lot 43, later transmitted dengue to other volunteers (A.V. 32–W and A.V. 34–S.). Comment: The feeding of one *A. albopictus*, lot 29, was followed in five days by typical dengue fever.

Experiment 5.—August 7, 1929. Ten *A. albopictus* of lot 29 fed on a volunteer (A.V. 24–H) who had previously been used in negative experiments July 11, 21, and 29, and had been observed for nine days prior to this test. After an incubation period of five and one-half days dengue fever developed August 12, 1929. The attack began abruptly with a chill, followed by fever, headache, generalized pains, and a flushing of the face. The typical symptoms, including pains and aches in the eyes and back, accompanied by leukopenia, persisted until August 15, 1929. Comment: The feeding of ten infected *A. albopictus*, lot 29, on this volunteer was followed after five days by typical dengue fever.

EXPERIMENTS WITH AËDES ALBOPICTUS, LOT 43

Fifty-four female normal *Aëdes albopictus*, which were descendants of the original stock bred in the laboratory, were infected August 12, 1929, by feeding on the blood of an experimental case of dengue (A.V. 27–P), during the first twelve hours of the disease. As this case of dengue had resulted from the bite of one mosquito of lot 29, the potentially infected group of lot 43 mosquitoes represented the transfer of the virus through a second lot of *A. albopictus*. The results are given in the records of experiments 6 and 7.

Experiment 6.—August 26, 1929. Twenty-two *A. albopictus* of lot 43 fed on a volunteer (A.V. 32–W) who had been used previously for a negative experiment August 17, 1929, and had been observed for nine days prior to this test. After an incubation period of five days, dengue fever developed August 31, 1929, beginning with headache, nausea, fever, and leukopenia. The disease, which lasted five days, was also characterized by generalized pains, nausea, vomiting, and dizziness. There was no rash. Comment: The feeding of twenty-two infected *A. albopictus* of lot 43 on this volunteer was followed after five days by typical dengue fever.

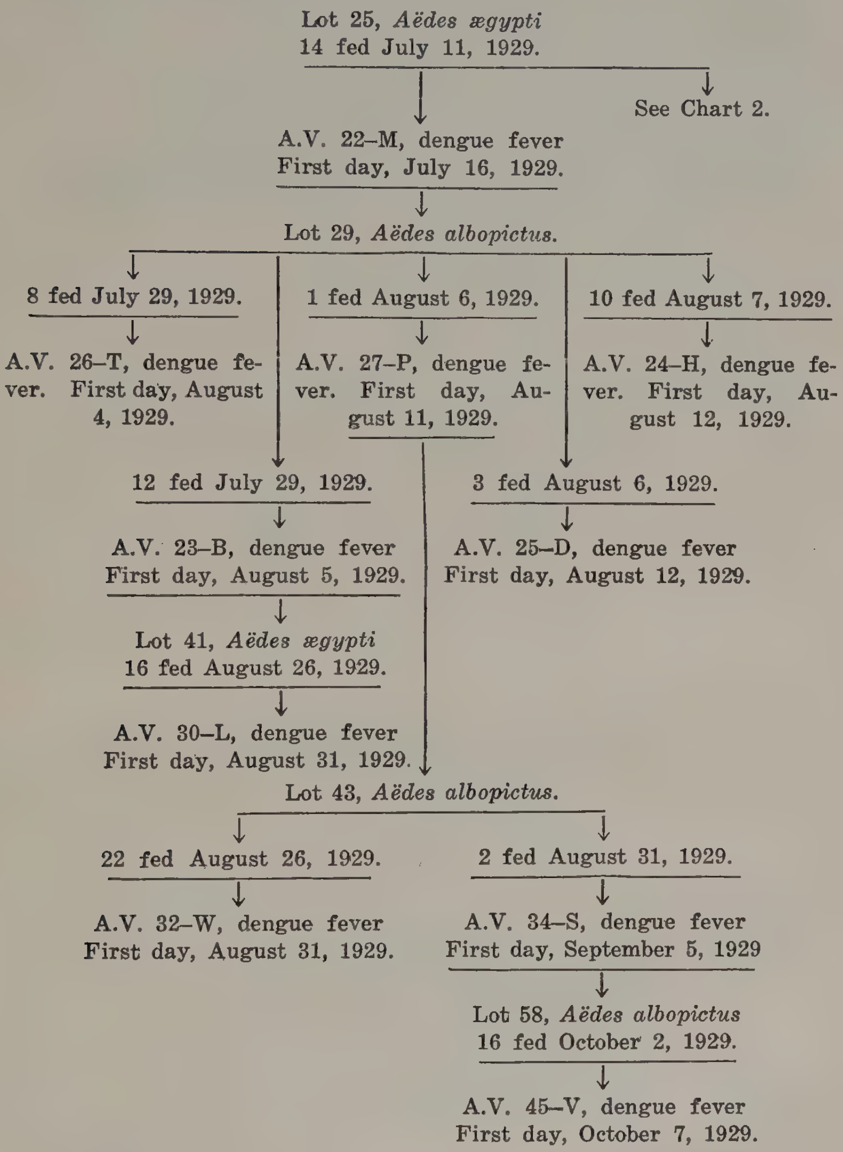
Experiment 7.—August 31, 1929. Two *A. albopictus* of lot 43 fed on a volunteer (A.V. 34–S) who had been admitted for this experiment alone, and had been observed for ten days prior to the test. After an incubation period of five days, typical dengue fever began September 5, 1929. The infection was characterized by headache, pains and aches in the eyes and muscles, weakness, dizziness, generalized rash, fever, and leukopenia. The duration of the disease was four and one-half days. Comment: The feeding of two infected *A. albopictus* of lot 43 on this volunteer was followed in eight days by typical dengue fever, proving that this mosquito is an effective dengue vector.

Experiment 8.—Sixteen normal *A. albopictus* were infected by feeding on the blood of American volunteer 34–S, September 6, 1929, and designated lot 58. October 2, 1929, three of these mosquitoes fed on A.V. 45–V and dengue fever developed October 7. The symptoms, which lasted for approximately four days, were typical, including a rash and leukopenia. Comment: Dengue fever developed in a susceptible individual following the feeding of three *A. albopictus* of lot 58.

CHART 1.—*Transfer of "K" strain of dengue virus from December 15, 1928, to July 16, 1929, by Aëdes ægypti and after that date by Aëdes albopictus, lots 29, 43, and 58.*

Keller, dengue fever
Second day, December 15, 1928.
↓
Lot 1, *Aëdes ægypti*
17 fed December 31, 1928.
↓
A.V. 1–H, dengue fever
First day, January 10, 1929.
↓
Lot 3, *Aëdes ægypti*
56 fed February 19, 1929.
↓
A.V. 3–A, dengue fever
First day, February 25, 1929.
↓
Lot 9, *Aëdes ægypti*
49 fed April 11, 1929.
↓
A.V. 6–R, dengue fever
First day, April 16, 1929.
↓
Lot 12, *Aëdes ægypti*
15 fed May 8, 1929.
↓
A.V. 9–D, dengue fever
First day, May 14, 1929.
↓
Lot 19, *Aëdes ægypti*
12 fed June 20, 1929.
↓
A.V. 14–A, dengue fever
First day, June 26, 1929.
↓
Lot 25, *Aëdes ægypti*.

CHART 1.—*Transfer of "K" strain of dengue virus from December 15, 1928, to July 16, 1929, by Aëdes ægypti and after that date by Aëdes albopictus, lots 29, 43, and 58*—Continued.


Lot 25, *Aëdes ægypti*
14 fed July 11, 1929.
See Chart 2.
A.V. 22–M, dengue fever
First day, July 16, 1929.
Lot 29, *Aëdes albopictus*.
8 fed July 29, 1929.
A.V. 26–T, dengue fever. First day, August 4, 1929.
1 fed August 6, 1929.
A.V. 27–P, dengue fever. First day, August 11, 1929.
10 fed August 7, 1929.
A.V. 24–H, dengue fever. First day, August 12, 1929.
12 fed July 29, 1929.
A.V. 23–B, dengue fever
First day, August 5, 1929.
3 fed August 6, 1929.
A.V. 25–D, dengue fever
First day, August 12, 1929.
Lot 41, *Aëdes ægypti*
16 fed August 26, 1929.
A.V. 30–L, dengue fever
First day, August 31, 1929.
Lot 43, *Aëdes albopictus*.
22 fed August 26, 1929.
A.V. 32–W, dengue fever
First day, August 31, 1929.
2 fed August 31, 1929.
A.V. 34–S, dengue fever
First day, September 5, 1929
Lot 58, *Aëdes albopictus*
16 fed October 2, 1929.
A.V. 45–V, dengue fever
First day, October 7, 1929.

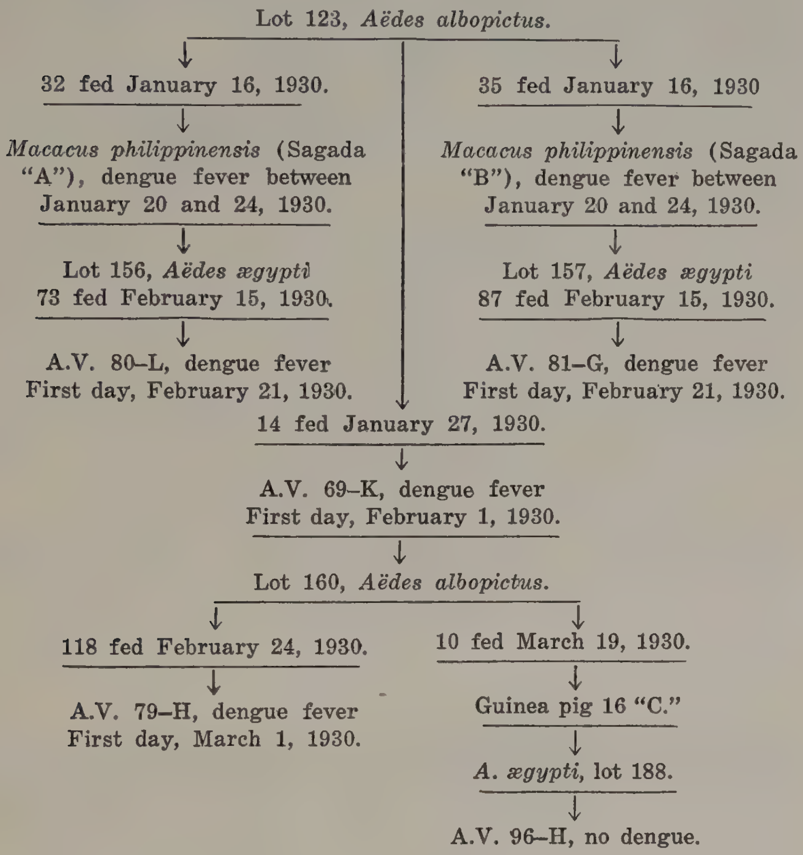
EXPERIMENTS WITH AËDES ALBOPICTUS, LOT 128

December 4, 1929, one hundred eighty-four normal female *A. albopictus* took infective blood from an experimental case of dengue (A.V. 62–H) during the second day of the disease. It is of interest to note here that the infection had been transferred to this volunteer through *A. ægypti*, lots 110–A, B, C, D, E, and F, from Japanese monkey (*Macacus fuscatus*) 2 (W).

CHART 2.—*Continuation of the passage of "K" strain of dengue virus through Aëdes ægypti from July 11, 1929, to November 28, 1929, and after that date by Aëdes albopictus of lots 128 and 160.*

Lot 25, *Aëdes ægypti*
15 fed July 11, 1929.
↓
A.V. 21–M, dengue fever
First day, July 17, 1929.
↓
Lot 32, *Aëdes ægypti*, combined with *A. ægypti* lots 35, 40, and 41
Fed to normal *A. ægypti* July 20, 1929.
↓
Lots 44, 45, 46, and 47 *Aëdes ægypti*
28 fed September 21, 1929.
↓
A.V. 36–V, dengue fever
First day, September 26, 1929.
↓
Lot 71, *Aëdes ægypti*
8 fed October 26, 1929.
↓
A.V. 55–S, dengue fever
First day, November 1, 1929.
↓ ↓
See Chart 3.
Blood inoculated.
↓
Macacus fuscatus 3 (X), dengue fever
between November 5 and 9, 1929.
↓
Lots 110–A, B, C, D, and E *Aëdes ægypti*
75 fed November 28, 1929.
↓
A.V. 62–H, dengue fever
First day, December 2, 1929.
↓
Lot 128, *Aëdes albopictus.*

CHART 2.—*Continuation of the passage of "K" strain of dengue virus through Aëdes ægypti from July 11, 1929, to November 28, 1929, and after that date Aëdes albopictus of lots 128 and 160*—Continued.


Lot 123, *Aëdes albopictus.*
32 fed January 16, 1930.
Macacus philippinensis (Sagada "A"), dengue fever between January 20 and 24, 1930.
Lot 156, *Aëdes ægypti*
73 fed February 15, 1930.
A.V. 80–L, dengue fever
First day, February 21, 1930.
35 fed January 16, 1930
Macacus philippinensis (Sagada "B"), dengue fever between January 20 and 24, 1930.
Lot 157, *Aëdes ægypti*
87 fed February 15, 1930.
A.V. 81–G, dengue fever
First day, February 21, 1930.
14 fed January 27, 1930.
A.V. 69–K, dengue fever
First day, February 1, 1930.
Lot 160, *Aëdes albopictus.*
118 fed February 24, 1930.
A.V. 79–H, dengue fever
First day, March 1, 1930.
10 fed March 19, 1930.
Guinea pig 16 "C."
A. ægypti, lot 188.
A.V. 96–H, no dengue.

These potentially infected *A. albopictus* were designated lot 128 and were later used to transmit dengue to two Philippine monkeys (*Macacus philippinensis*) and to one human volunteer as described in the following experiments.

Experiment 9.—January 16, 1930. Thirty-two *A. albopictus* of lot 128 fed on a Philippine monkey *M. philippinensis* 30 (Sagada "A"). This monkey had been obtained from the mountains of northern Luzon and observed in a screened cage for a period of about two weeks prior to this test. While the monkey failed to show any recognizable symptoms of dengue, groups of normal *A. ægypti,* which fed on the animal January 20, 21, 22, 23, and 24 and were designated lot 156 later transmitted dengue (February 15, 1930) to a susceptible human volunteer (A.V. 80–L). Comment: The observations made in this experiment show that thirty-two *A. albopictus* of lot 128 produced dengue in a Philippine monkey.

Experiment 10.—January 16, 1930. Thirty-five *A. albopictus* of lot 128 fed on *M. philippinensis* 31 (Sagada "B"), which had been obtained from the mountains of northern Luzon and observed in a screened cage for a period of about two weeks prior to this test. While this monkey also failed to show any recognizable signs of dengue, it is apparent that infection occurred, since groups of normal *A. ægypti* fed on the animal January 20, 21, 22, 23, and 24, and designated lot 157, later transmitted dengue (February 15, 1930) to a susceptible human volunteer (A.V. 81–G). Comment: The observations made in this experiment show that the feeding of thirty-five *A. albopictus* of lot 128 produced dengue in a Philippine monkey.

Experiment 11.—January 27, 1930. Fourteen *A. albopictus* of lot 128 fed on a volunteer (A.V. 69–K) who had been used previously for a negative experiment January 13 and had been observed for fourteen days prior to this test. After an incubation period of five days, typical dengue fever developed at noon February 1, beginning with a chill followed by fever. The symptoms, which persisted for about five and one-half days, included headache, pain in various parts of the body, glandular enlargement, weakness, dizziness, fever, leukopenia, and a terminal rash. Comment: The feeding of fourteen infected *A. albopictus* of lot 128 on this volunteer was followed in five days by typical dengue fever.

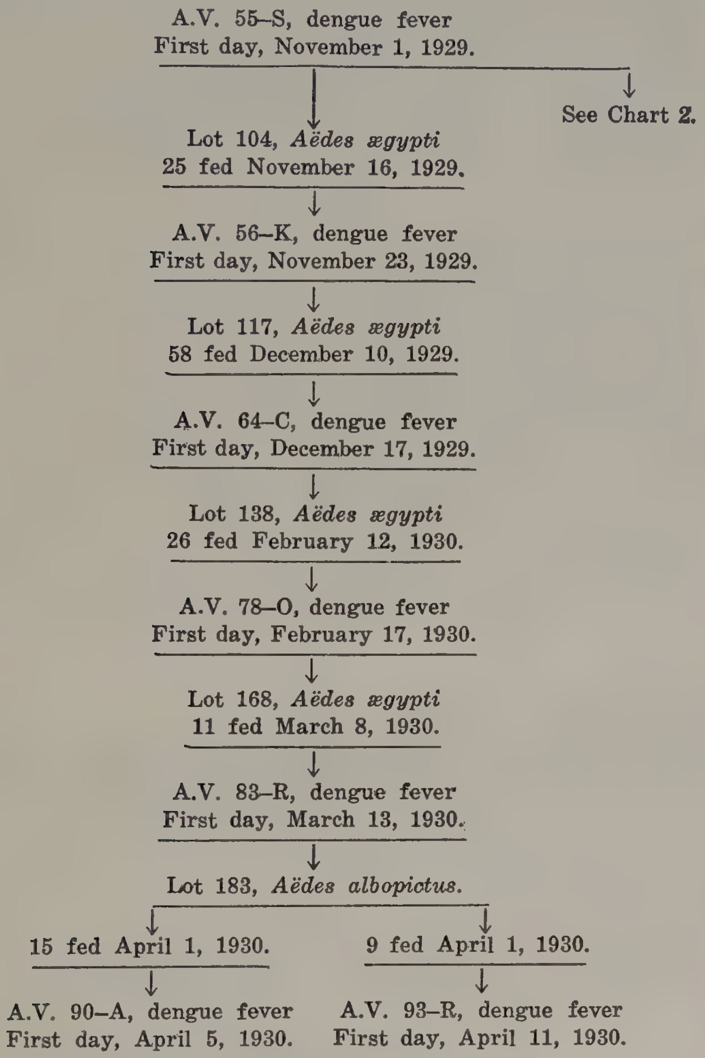
EXPERIMENTS WITH AËDES ALBOPICTUS, LOT 160

February 2, 1930, two hundred fifty normal *A. albopictus* took infective blood from an experimental case of dengue (A.V. 69–K) during the first day of the disease. These mosquitoes, designated lot 160, were later fed on one human volunteer and one guinea pig, as described in experiments 12 and 13.

Experiment 12.—February 24, 1930. One hundred eighteen *A. albopictus* of lot 160 fed on a volunteer (A.V. 79–H) who had been used previously for a negative experiment February 15, 1930, and had been observed for eight days prior to this test. After an incubation period of five days, typical dengue fever developed March 1, 1930, beginning with fever, nausea, and vomiting. The symptoms continued for about five days and were characterized by both primary and terminal rashes, fever, leukopenia, nausea, dizziness, headache, pains in eyes, back, chest, and joints, and some glandular enlargement. Comment: The feeding of one hundred eighteen *A. albopictus* of lot 160 on this volunteer was followed in five days by typical dengue fever.

Experiment 13.—March 19, 1930. Ten *A. albopictus* of lot 160 fed on a young guinea pig "C," which had been born in a mosquito-proof cage and kept there until the time of this test. Normal *A. ægypti* (lot 188) were fed on this guinea pig daily during the fifth to eighth days, inclusive, after it had been bitten by *A. albopictus* (lot 160). Six and twelve *A. ægypti* of lot 188 were tested on dengue-susceptible volunteer A.V. 96–H. Dengue did not develop. Comment: Either the guinea pig was immune or the mosquitoes, fed on the day when the virus was infective, had died before they were tested on the volunteer. This experiment was considered inconclusive.

CHART 3.—*Continuation of passage of "K" strain of dengue virus through Aëdes ægypti from November 1, 1929, to March 13, 1930, and after that date by Aëdes albopictus of lot 183.*


A.V. 55–S, dengue fever
First day, November 1, 1929.
See Chart 2.
Lot 104, *Aëdes ægypti*
25 fed November 16, 1929.
A.V. 56–K, dengue fever
First day, November 23, 1929.
Lot 117, *Aëdes ægypti*
58 fed December 10, 1929.
A.V. 64–C, dengue fever
First day, December 17, 1929.
Lot 138, *Aëdes ægypti*
26 fed February 12, 1930.
A.V. 78–O, dengue fever
First day, February 17, 1930.
Lot 168, *Aëdes ægypti*
11 fed March 8, 1930.
A.V. 83–R, dengue fever
First day, March 13, 1930.
Lot 183, *Aëdes albopictus.*
15 fed April 1, 1930.
9 fed April 1, 1930.
A.V. 90–A, dengue fever
First day, April 5, 1930.
A.V. 93–R, dengue fever
First day, April 11, 1930.

EXPERIMENTS WITH AËDES ALBOPICTUS, LOT 183

March 13, 1930, one hundred sixteen normal *A. albopictus* took infective blood from an experimental case of dengue (A.V. 83–R) during the first day of the disease. These mosquitoes, designated lot 183, were later used in transmission experiments with two human volunteers as described in experiments 14 and 15.

Experiment 14.—April 1, 1930. Fifteen *A. albopictus* of lot 183 fed on a volunteer (A.V. 90–A) who had been used previously for a negative experiment March 22, 1930, and had been observed for nine days prior to this test. After an incubation period of four and one-half days, dengue fever developed April 5, 1930.

Experiment 15.—April 1, 1930. Nine *A. albopictus* of lot 183 fed on a volunteer (A.V. 93–R) who had been used previously for a negative experiment March 22, 1930, and had been observed for nine days prior to this test. After an incubation period of ten days dengue fever developed April 11, 1930.

DISCUSSION

The results of these experiments prove that *Aëdes albopictus,* a common Oriental mosquito, is an effective dengue carrier, and make it necessary to reconsider the available evidence concerning the epidemiological importance of this species. From the observations made by the board and by others who have studied the mosquito, it is apparent that *A. albopictus* somewhat resembles *A. ægypti* in its choice of breeding places and in its habit of feeding on human beings mainly during the day. However, the former mosquito may require a few more days for development to the adult stage, and appears to be even more vicious and daring in biting man. The geographical distribution of *A. albopictus* given by Theobald,(5) Barraud,(6) and others indicates that this species is prevalent in many localities, including India, Formosa, the Philippine Islands, and other parts of the Orient where dengue is endemic. Cleland(8) and others mention the fact that *A. albopictus* has been suspected as a dengue vector on epidemiological grounds alone; while this mosquito was under grave suspicion during the 1915 dengue epidemic in Formosa.(7) Furthermore, observations made in Manila during 1929 indicate that this mosquito was especially prevalent during June and July, at the time of the highest incidence of dengue fever. From the evidence presented in this report, it is obvious that *A. albopictus* must be considered seriously as a factor in the dissemination of dengue fever.

SUMMARY AND CONCLUSIONS

1. Collections of larvæ and of adult mosquitoes from representative parts of the city indicate that *Aëdes albopictus* Skuse, like *A. ægypti,* is a common species in Manila, Philippine Islands.
2. A review of the literature indicates that *A. albopictus* is prevalent not only in the Philippines but throughout the Orient, and that this mosquito has been suspected as a possible vector of dengue.

TABLE 4.—*Transmission of dengue to man and monkeys by Aëdes albopictus.*

Experiment No.	Infective feeding.					
	Lot No.	Number fed.	Date fed.	Time fed.	Source of virus.	Day of dengue.
				a. m.		
1	29	80	July 16, 1929	10	A.V. 22–M	First.
2						
3						
4						
5						
6	43	54	Aug. 11, 1929	11.30	A.V. 27–P	Do.
7						
8	58	16	Sept. 6, 1929	9.30	A.V. 34–S	Do.
9	128	184	Dec. 4, 1929	11	A.V. 62–H	Second.
10						
11						
12	160	250	Feb. 2, 1930	9	A.V. 69–K	First.
14	183	116	Mar. 13, 1930	10.30	A.V. 83–R	Do.
15						

Experiment No.	Subsequent use.				
	Number fed.	Date fed.	Time fed.	On whom fed.	Onset of dengue.
			a. m.		
1	12	July 29, 1929	10	A.V. 23–B	a. m. Aug. 5, 1929.
2	8	July 29, 1929	10	A.V. 26–T	p. m. Aug. 4, 1929.
3	3	Aug. 6, 1929	11.30	A. V. 25–D	p. m. Aug. 12, 1929.
4	1	Aug. 6, 1929	11.30	A.V. 27–P	a. m. Aug. 11, 1929.
5	10	Aug. 7, 1929	11.15	A.V. 24–H	a. m. Aug. 12, 1929.
6	22	Aug. 26, 1929	10	A.V. 32–W	a. m. Aug. 31, 1929.
7	2	Aug. 31, 1929	10	A.V. 34–S	a. m. Sept. 5, 1929.
8	3	Oct. 2, 1929	11	A.V. 45–V	Sept. 7, 1929.
9	82	Jan. 6, 1930	9–11	*Macacus philippinensis* 30 (Sagada "A").	Between Jan. 20 and 24, 1929.[a]
10	35	Jan. 16, 1930	9–11	*Macacus philippinensis* 31 (Sagada "B").	Between Jan. 20 and 24, 1929.[a]
11	14	Jan. 27, 1930	10	A.V. 69–K	Feb. 2, 1930.
	8	Feb. 7, 1930	9	A.V. 72–E	(To test immunity.)
12	118	Feb. 24, 1930	10.30	A. V. 79–H	Mar. 1, 1930.
14	15	Apr. 1, 1930	9.30	A.V. 90–A	April 5, 1930.
15	9	Apr. 1, 1930	9.30	A.V. 93–R	April 11, 1930.

[a] Infection was proved in these monkeys by transferring the virus from them through *A. ægypti,* lots 156 and 157 to susceptible volunteers A.V. 80–L., A.V. 81–G, and A.V. 69–K.

3. During these experiments the transmission of dengue by this mosquito to twelve American soldiers and to two Philippine monkeys (*Macacus philippinensis*) proves that *A. albopictus* is an effective carrier of dengue.

4. In these successful transmission experiments with *A. albopictus* the intervals between the ingestion of infective blood and the tests on volunteers were from thirteen to fifty-four days.

5. Dengue resulted in the different experiments from the feeding of 1, 2, 3, 8, 8, 9, 10, 12, 14, 15, 22, 32, 35, and 118 infected *A. albopictus*, respectively.

6. *Aëdes albopictus* must be considered as an important agent in the dissemination of dengue.

7. In view of the fact that two distinct species of *Aëdes* are now known to be dengue vectors, it is reasonable to suspect that still others may be concerned in the spread of this disease.

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DENGUE TRANSMISSION EXPERIMENTS WITH CULEX QUINQUEFASCIATUS

Culex quinquefasciatus, formerly known as *Culex fatigans,* was the first mosquito to be incriminated as a dengue vector. Observations of early workers concerning the similarity between the distribution of dengue and the geographical distribution of this mosquito led to its use in transmission experiments. Graham,(1) who first established the fact that dengue can be transferred to man by mosquitoes, reported several successful experiments with *C. fatigans.* In his work, mosquitoes which had been captured inside the netting covering dengue patients were immediately placed inside the netting covering normal persons. Dengue occurred in three of four experimental volunteers after incubation periods of four, five, and six days. While the volunteers had agreed to remain in their homes throughout the experiments, Graham was apparently not sure on this point, as he made the following comment: "I still felt that these three men might have received the contagion in some other way." He then collected mosquitoes from inside the netting of a dengue patient, took them to a dengue-free village at an elevation of 3,000 feet, and gave them an opportunity to feed on two young men who lived in different parts of the town. After incubation periods of four and five days, respectively, both of these men developed dengue. No other case occurred in the village. Since there was only a short interval between the first and second stages of these experiments, it seems possible that in some instances an interrupted type of feeding might have occurred, resulting in a mechanical transfer of dengue-infected blood to the volunteers. However, uncertainty about the possibility of having included some "Stegomyia" with the lots of *Culex* used, has led to doubt concerning the identity of the mosquitoes responsible for the infections in his experiments.

Stimulated by Graham's observations and by the fact that this mosquito was commonly found in endemic dengue localities in the Philippines, Ashburn and Craig(2) (1907) used *Culex fatigans,* raised from the egg in their laboratory, in a series of transmission experiments. The procedure followed was to liberate the mosquitoes at night beneath the net covering a dengue patient isolated in a mosquito-proof tent. On the following morning the mosquitoes were collected and liberated inside a mosquito bar covering the experimental dengue volunteer who remained beneath the net until all the mosquitoes had disappeared. Four of nine volunteers exposed in this manner were satisfactorily bitten, and one of these four developed typical dengue. It was believed that this individual had not been exposed to dengue for some time prior to the experiment, and he was not allowed to leave the mosquito-proof covering until after the disease began. In this one successful experiment the mosquitoes which had fed on a patient September 11, 1906, were placed in the net with the volunteer on the following day, September 12, and dengue developed a little over four days later.

Cleland, Bradley, and McDonald(3) in 1916 performed four experiments using a slightly different technic in which they failed to transmit dengue with *Culex fatigans*. In the first series mosquitoes collected in the rooms of dengue patients April 3, 4, and 6, 1916, were given an opportunity to feed on two volunteers, "Mac" and "Joe," April 8, 9, and 11. Four mosquitoes took blood from "Mac" on the evening of April 8, while two, "several," and one bit J. O. S. on the respective days. Dengue did not develop. In the second series of experiments a volunteer "M" was bitten by twelve of these mosquitoes May 11, and by an unknown number May 12 and 13, and by at least twenty May 14. Then after adding fifty-three *Culex*, some of which had been caught in the houses of dengue patients, the lot was given access to a laboratory assistant, J. O. S., who was bitten by two mosquitoes May 15 and by —— May 18, 1916. Both of these men remained well. In discussing these four experiments the authors made the following comment: "We have not in our opinion shown conclusively that *Culex fatigans* may not also spread the disease though we think this unlikely." They also remarked concerning the experiments of Ashburn and Craig: "If their successful case arose from the bites of *Culex fatigans* and was not a case of natural infection, the most reasonable view to take is that in this instance the mosquitoes merely acted as infected lancets and not as true intermediate hosts." Siler, Hall, and Hitchens(4) in 1924 tried to infect seven susceptible volunteers with laboratory-bred *Culex quinquefasciatus*, which twelve to twenty-two days previously had fed on patients during the infective stage of dengue. The results were negative, while *A. ægypti,* which had been fed on infected blood from the same sources and later fed on the same volunteers used for the *Culex* experiments, produced dengue in each instance. Blanc and Caminopetros(5) in 1928 reported failure to transmit dengue with *Culex* mosquitoes, including *C. pipiens, C. apicalis,* and *C. laticinctus,* during experiments in which, after feedings of infective blood, the mosquitoes were kept five, ten, fifteen, eighteen, twenty-two, and thirty-four days before they were tested for infectivity.

In the present study the experiments were planned with a view to determining whether or not *C. quinquefasciatus* becomes infected after a feeding of dengue blood; and, if infected, whether or not it is able to transmit the disease to man after a period similar to that required by *A. ægypti.*

EXPERIMENTAL

Source of mosquitoes.—The two lots of *Culex quinquefasciatus* (Nos. 31 and 133) used in these experiments were selected from mosquitoes that emerged from larvæ collected in Manila and raised in the laboratory. Each mosquito conformed to the description of *C. quinquefasciatus* given by Dyar.(6)

Source of dengue virus.—The blood fed to the mosquitoes of these lots contained infective dengue virus. Lot 31: July 16, 1929, between 7 and 9 p. m., one hundred seven normal *C. quinquefasciatus* took full feedings of infective blood from an experimental case of dengue (A.V. 21–M), seven hours after the onset of the disease. The infectivity of the blood at this time was indicated by the fact that normal *A. ægypti,* which fed on the patient at 9.15 a. m., July 17 (lot 32), later transmitted dengue to a volunteer (A.V. 28–P). The *Culex* mosquitoes fed on this case and designated lot 31 were later used in experiments 1, 2, 3, 4, and 5. Lot 133: December 4, 1929, between 7 and 10.30 p. m., fifty-two normal *C. quinquefasciatus* took full feedings of blood from an experimental case of dengue (A.V. 60–S) during the first day of the disease. These mosquitoes were used in experiment 6.

Experiment 1.—Attempt to determine whether or not infective virus remains in Culex quinquefasciatus four days after the ingestion of dengue blood. July 20, 1929, 1.15 p. m. Twenty-five *C. quinquefasciatus* of lot 31, which had *fed on infective blood four days previously* were killed, macerated with sterile sand in a porcelain mortar, suspended in physiologic salt solution, and filtered through a preliminary type of Mandler filter. Three cubic centimeters of the filtrate, representing about eighteen of the mosquitoes, was injected subcutaneously into a susceptible volunteer (A.V. 28–P). Dengue failed to develop from this inoculation. Comment: The evidence was not considered sufficient to prove that these mosquitoes were not infected, because similar negative results were also obtained at times even with filtrates obtained from suspensions of *A. ægypti* known to be infective. Therefore, no definite conclusion could be drawn from this experiment.

The next four experiments were performed in order to determine the ability of *C. quinquefasciatus* to transmit dengue by biting thirteen, thirty-three, and forty-five days after they had ingested infective blood.

Experiment 2.—July 29, 1929, 7 to 9 p. m. Seven *C. quinquefasciatus* of lot 31, *which had taken infective blood thirteen days previously,* fed on a susceptible volunteer (A.V. 24–H). Dengue did not develop. August 7 this volunteer was tested for susceptibility with an infective lot of *A. albopictus* (No. 29), and developed dengue August 12. Comment: In this experiment *C. quinquefasciatus,* which had ingested infective blood thirteen days previously, failed to transmit dengue by biting.

Experiment 3.—July 29, 1929, 7 to 9 p. m. Four *C. quinquefasciatus* of lot 31, *which had taken infective blood thirteen days previously,* fed on a susceptible volunteer (A.V. 25–D). Dengue did not develop in thirteen days. This volunteer was tested for susceptibility with an infective lot of *A. albopictus* (No. 29) August 6, and developed dengue August 12, 1929. Comment: This experiment indicates that *C. quinquefasciatus,* which had ingested infective blood thirteen days previously, failed to transmit dengue.

Experiment 4.—August 18, 1929, 6 to 9 p. m. Ten *C. quinquefasciatus* of lot 31, *which had ingested infective blood thirty-three days previously,* fed on a susceptible volunteer (A.V. 31–D). Dengue did not develop in eleven days. This volunteer was tested for susceptibility with an infective lot of *A. ægypti* (No. 35) August 26, and developed dengue four days later, August 30, 1929. Comment: As the interval between the feeding of *Culex* on this volunteer and the beginning of dengue was longer than the usual incubation periods observed in dengue, there was no reason to suppose that the infection was due to *Culex,* particularly since infective *A. ægypti,* lot 35, had fed in the meantime. However, in the next experiment it was planned to observe the volunteer for a longer period before applying the test for susceptibility.

Experiment 5.—August 30, 1929, 6 to 9 p. m. Five *C. quinquefasciatus, which had taken infective blood forty-five days previously,* fed on a susceptible volunteer (A.V. 33–B). Dengue did not develop in eighteen days. This volunteer was purposely held for a longer period than usual before applying the test for susceptibility, in order to eliminate the possibility of infection after a prolonged incubation period. The test for susceptibility was made September 14 with an infective lot of *A. ægypti* (No. 49), and dengue followed after an incubation period of five days, September 18, 1929. Comment: In this experiment *C. quinquefasciatus,* which had ingested infective blood forty-five days previously, failed to transmit dengue by biting.

Experiment 6.—To determine whether infective virus remains in Culex quinquefasciatus fourteen days after the ingestion of dengue blood. December 18, 1929. Thirty-eight *C. quinquefasciatus* of lot 133 were macerated, mixed with 2.5 cubic centimeters of a normal guinea pig's blood, placed in a shallow cell under a piece of chicken skin, and fed to one hundred forty normal *A. ægypti.* The latter mosquitoes, designated *A. ægypti* lot 144, were tested after thirty-five days by feeding on a susceptible volunteer (A.V. 75–P), who did not develop dengue during an observation period of sixteen days. February 8 dengue fever developed from the feeding of dengue-infected *A. ægypti* of lot 138, February 3, 1930. Comment: As normal *A. ægypti* may be infected with dengue virus by feeding on similarly prepared suspensions of infected *Aëdes*, it is believed that the negative results obtained in this experiment indicate that *C. quinquefasciatus* of lot 133 were not properly infected fourteen days after they had ingested blood from a dengue patient.

DISCUSSION

Groups of *Culex quinquefasciatus* which had fed on infective blood thirteen to forty-five days previously failed to transmit dengue when tested on susceptible volunteers. Similar negative observations have been reported by Siler, Hall, and Hitchens(4) and by others who have investigated the possibility of dengue transmission by this mosquito under conditions similar to those required for *A. ægypti.*

Attempts were also made to determine the length of time during which dengue virus remains alive after ingestion by *Culex*, and to determine whether this mosquito may become permanently infected as does *A. ægypti*, but is unable to transmit the virus to man during feeding because of some physiological or anatomical peculiarity. At first it was considered probable that these questions might be answered by injecting susceptible volunteers with bacteria-free filtrates of suspensions of *Culex* killed at different intervals after they had fed on blood containing infective dengue virus. However, because of the irregular results obtained in filtration experiments with suspensions of *A. ægypti* known to have been infective, the method was abandoned as unreliable. The later development of a technic by which normal *A. ægypti* were infected with dengue virus by feeding on suspensions of infective *A. ægypti* in normal blood, led to the use of a similar technic for testing *C. quinquefasciatus* for infectivity. Such experiments were begun with two groups of *Culex*, which had fed on dengue blood twenty-four hours and fourteen days previously. Unfortunately the former experiment was not completed, due to loss of the *A. ægypti* that fed on the suspension of *Culex* which had ingested blood containing dengue virus twenty-four hours previously. However, the latter experiment (No. 6) was carried through satisfactorily. The result was negative, indicating that either dengue virus was not present in the *Culex* mosquitoes used in the suspension or, if present, the amount was so small or the virus so attenuated that the infection could not be transferred through *A. ægypti* to man. Whatever the real explanation, it is apparent that *C. quinquefasciatus* may feed on a case of dengue during the infective stage of the disease and again feed on susceptible individuals thirteen to forty-five days later without transmitting dengue.

These observations naturally have no application to the possibility of transferring dengue virus mechanically by *Culex* or other blood-sucking insects that are interrupted while feeding on infective patients and immediately afterwards complete their meal of blood on susceptible individuals. The subject of mechanical transmission is discussed in a separate chapter.

SUMMARY AND CONCLUSIONS

1. In four experiments *C. quinquefasciatus,* which had fed on infective blood thirteen to forty-five days previously, failed to transmit dengue by biting susceptible volunteers.
2. A volunteer injected subcutaneously with the "Mandler" filtrate of a saline suspension of *C. quinquefasciatus,* which four days previously had fed on infective dengue blood, failed to develop dengue.
3. Normal *A. ægypti,* fed on a suspension of *C. quinquefasciatus* fourteen days after the latter had ingested dengue blood, failed to transmit dengue to a susceptible volunteer. Therefore, it seems probable that within fourteen days or less after *C. quinquefasciatus* has ingested infective blood the virus in this mosquito either dies or loses its ability to infect either man or *Aëdes* mosquitoes.
4. If *C. quinquefasciatus* is able to transmit dengue to man it is believed that this probably occurs as a result of mechanical transfer of infected blood during interrupted feeding.

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MECHANICAL TRANSMISSION OF DENGUE VIRUS THROUGH "INTERRUPTED FEEDING" BY CULEX QUINQUEFASCIATUS AND AËDES ÆGYPTI

It is not unusual for mosquitoes interrupted while feeding on one individual to attempt to complete their meal of blood on the same person or on others. That mosquitoes are rarely allowed to take a complete feeding of blood from a single person cannot be questioned, as the discomfort associated with the insertion of the proboscis usually leads to attempts to destroy the insect, an act which is often unsuccessful and usually results only in interrupting the feeding. While the mosquito may attempt to feed again on the same individual, it may attack some other person. Thus, if an organism of sufficient virulence should be present in the blood of the first individual bitten, it is not unreasonable to suspect that in some instances it might be carried mechanically on or in the proboscis of the mosquito to other individuals and that in this way it might be possible to transmit certain blood infections mechanically. The possibility of such mechanical transmission of dengue by mosquitoes has been suggested and discussed by various investigators; while Graham(1) and Ashburn and Craig(2) have reported positive experiments with *Culex quinquefasciatus*, in which dengue transmission apparently resulted from interrupted feeding of the mosquitoes on dengue patients followed immediately by refeeding on susceptible persons.

EXPERIMENTAL

The possibility of transmitting dengue in this manner was investigated by allowing *Culex quinquefasciatus* and *Aëdes ægypti* to partially fill with infective blood from experimentally induced cases of dengue, after which the mosquitoes were immediately transferred to susceptible human volunteers and allowed to complete the interrupted feedings.

MECHANICAL TRANSMISSION OF DENGUE VIRUS BY CULEX QUINQUEFASCIATUS

Experiment 1.—A susceptible volunteer (A.V. 54–K) admitted October 21, 1929, was observed in a screened cubicle for ten days before the experiment. October 31, 1929, between 9 and 10.30 p. m., one hundred normal *Culex quinquefasciatus*, designated lot 97, were permitted to take partial feedings of infective blood from an experimental case of dengue (A.V. 55–S) during the first day of the disease. These mosquitoes were immediately transferred to the susceptible volunteer (A.V. 54–K) on whom fifty-four of them resumed feeding and became completely filled with blood. After an incubation period of eleven and one-half to thirteen days this volunteer developed dengue fever. The temperature reached 99.4° F. at noon November 12, while the patient first complained of illness on the evening of November 13. The symptoms as noted in the protocol of this volunteer were typical of dengue. Comment: In this experiment dengue apparently was transmitted to man mechanically through interrupted feeding by *C. quinquefasciatus*.

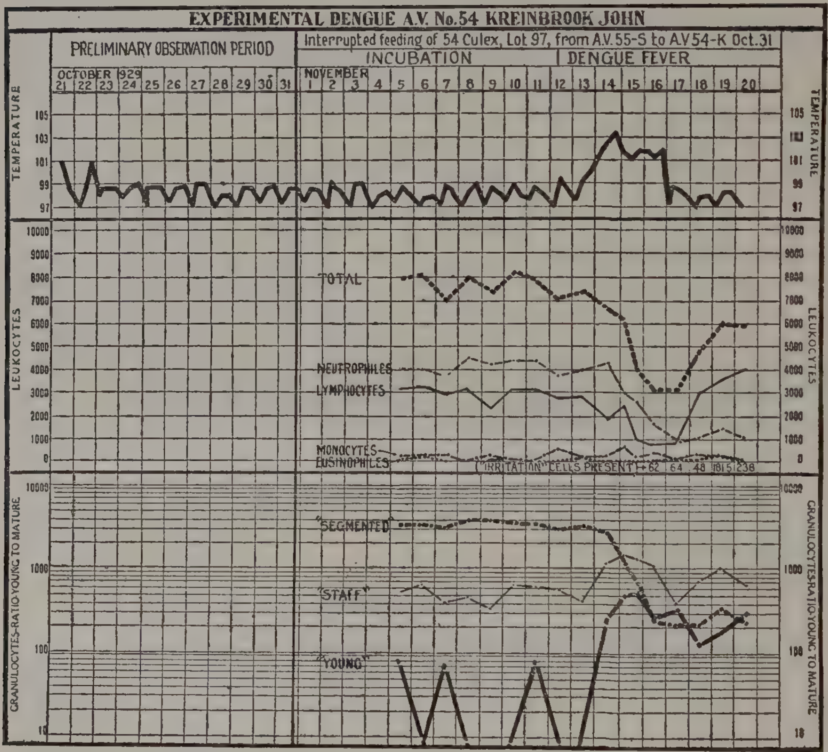

FIG. 10. Experimental dengue in American volunteer 54.

As the positive result obtained in the first experiment indicated that dengue virus might be transferred mechanically when large numbers of mosquitoes took part in the feeding, the following experiments were performed to determine whether similar results might be obtained when small numbers of mosquitoes were employed.

Experiment 2.—December 4, 1929, 6.30 to 7.30 p. m. Eight *C. quinquefasciatus* called lot 132, which had partially filled with infective blood from A.V. 60–S during the first day of dengue, immediately completed feeding on a susceptible volunteer (A.V. 58–S). This man did not develop dengue during fifteen days of subsequent observation in the ward. Because of the Christmas holidays, the volunteer was allowed to leave the ward before a test was made to determine his susceptibility to dengue, and it was not possible to do this until three months later. No history could be obtained of any illness during this period. March 31, 1930, twenty-six infected *A. ægypti* fed on him, after which he remained well for a period of at least two weeks and, therefore, was considered to be immune to dengue. Comment: The results of this experiment were inconclusive, due to the fact that the volunteer used to test the infectivity of the mosquitoes was immune.

Experiment 3.—February 21, 1930. Twenty-one of twenty-six *C. quinquefasciatus* called lot 170, which had partially filled with infective blood from A.V. 80–L between 6.30 and 9.30 p. m. on the first day of dengue, completed feeding before 10.30 p. m. on a susceptible volunteer (A.V. 82–F). Dengue did not develop during an observation period of eighteen days; however, after one hundred eight infective *A. ægypti* of lot 167 had fed March 4, dengue followed March 11, 1930. Comment: In this experiment twenty-one *C. quinquefasciatus*, which had partially fed on dengue blood and immediately refed on a susceptible volunteer, failed to transmit dengue.

Experiment 4.—March 13, 1930. Twelve of twenty-six normal *C. quinquefasciatus*, designated lot 185, which had taken partial feedings of infective blood from a patient (A.V. 83–R) between 7 and 10.30 p. m. on the first day of dengue, completed feeding within an hour on a susceptible volunteer (A.V. 85–T). Dengue did not develop during eighteen days observations. Infected *A. ægypti* of lot 164 were fed March 26, and after six and one-half days dengue occurred April 1, proving that the volunteer was not immune. Comment: In this experiment the refeeding of twelve *C. quinquefasciatus*, immediately after they had ingested dengue blood, failed to cause the disease in a susceptible volunteer.

Experiment 5.—March 13, 1930. Eight of twenty-six *C. quinquefasciatus* called lot 185, which had taken infective blood from a patient (A. V. 83–R) between 7 and 10.30 p. m. on the first day of dengue, completed feeding between 9 and 11 p. m. on a susceptible volunteer (A.V. 86–B). This man remained well for seventeen days. However, after being bitten by twenty-three *A. ægypti*, lot 165, March 26, dengue developed March 31. Comment: In this experiment the refeeding of eight *C. quinquefasciatus* within a few minutes after they had ingested infective dengue blood failed to cause the disease in a susceptible volunteer.

MECHANICAL TRANSMISSION OF DENGUE VIRUS BY AËDES ÆGYPTI

Experiment 6.—March 24, 1930. Two hundred normal *A. ægypti*, called lot 189, which at 10.30 a. m. had taken partial feedings of infective blood from a volunteer (A.V. 87–R) during the first day of dengue, were transferred immediately to a susceptible volunteer (A. V. 94–K). More than one hundred twenty-five of the mosquitoes refed and became filled with blood. After an incubation period of seven to nine days this volunteer developed dengue fever. Comment: In this experiment dengue was apparently transmitted to man through the interrupted feedings of *A. ægypti*.

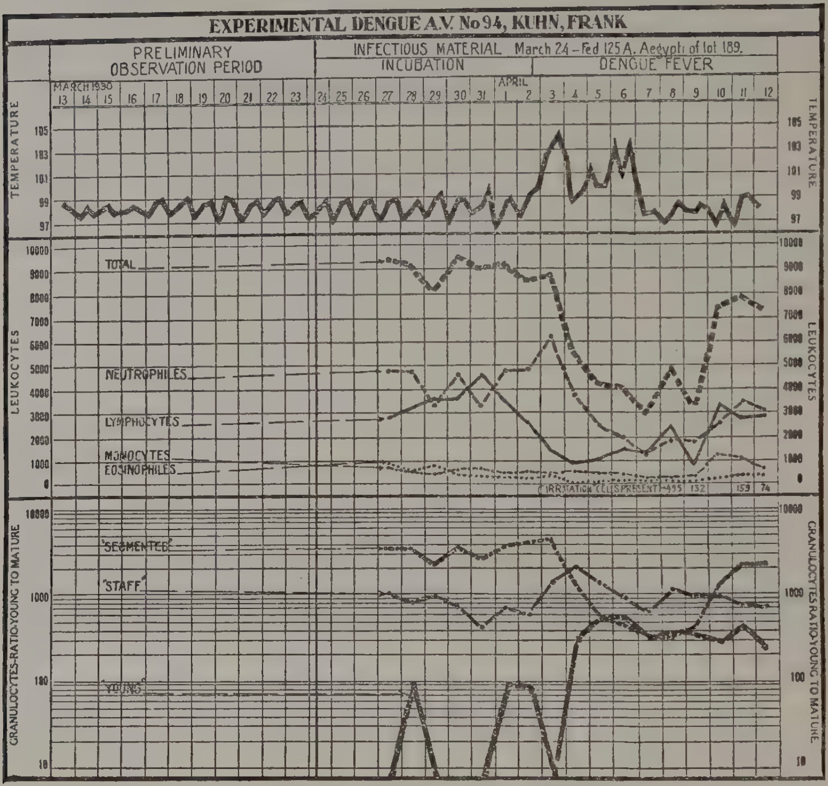

FIG. 11. Experimental dengue in American volunteer 94.

DISCUSSION

The results obtained in these experiments indicate that during the infective period of dengue fever, virus may be carried from patients to susceptible individuals by immediate mechanical transfer on or in the proboscis of *C. quinquefasciatus* or *A. ægypti*. However, such transfer of virus occurred only in experiments 1 and 6, in which relatively large numbers of mosquitoes, fifty-four and one hundred twenty-five, respectively, completed their feedings on susceptible volunteers; while in similar experiments performed with eight, twelve, and twenty-

TABLE 5.—*Mechanical transmission of dengue virus by interrupted feedings of Culex quinquefasciatus and Aëdes ægypti.*

Experiment No.	Date.	Mosquitoes allowed partial feedings of infective blood.					
		Experimental case of dengue.		Mosquitoes that took blood.			
		Volunteer.	Day of the disease.	Species.	Lot No.	Number fed.	Time fed.
1	Oct. 31, 1929	A.V. 55-S	First	*Culex quinquefasciatus.*	97	100	9-10.30 p. m.
2	Dec. 4, 1929	A.V. 60-S	do	do	132	8	6.30-7.30 p. m.
3	Feb. 21, 1930	A.V. 80-L	do	do	170	26	6.30-9.30 p. m.
4	Mar. 13, 1930	A.V. 83-R	do	do	185	26	7-10.30 p. m.
5	Mar. 13, 1930	A.V. 83-R	do	do	185	26	7-10.30 p. m.
6	Mar. 24, 1930	A.V. 87-R	do	*Aëdes ægypti*	189	160	10.30 a. m.

Experiment No.	Date.	Same mosquitoes refed on susceptible volunteers.			
		Time fed.	Number fed.	Volunteer.	Results.
1	Oct. 31, 1929	10.30-11.30 p. m.	54	A.V. 54-K	Dengue fever (in 11½ to 13 days).
2	Dec. 4, 1929	6.45-8 p. m.	8	A.V. 58-S	Immune.
3	Feb. 21, 1930	6.45-10.30 p. m.	21	A.V. 82-F	Negative.
4	Mar. 13, 1930	8-11 p. m.	12	A.V. 85-T	Do.
5	Mar. 13, 1930	8-11.00 p. m.	8	A.V. 86-B	Do.
6	Mar. 24, 1930	10.45 a. m.	125	A.V. 94-K	Dengue fever (in 7 to 9 days).

one mosquitoes the results were negative. These results indicate either that many individual mosquitoes failed to mechanically carry infective blood or virus; or that each mosquito used carried to the volunteer such minute amounts of the patient's blood that large numbers of mosquitoes feeding simultaneously were necessary in order to transfer sufficient virus to cause dengue infection in man. From the rather long incubation period it may be suspected that even in the successful experiments only small amounts of dengue virus were injected into the volunteers. For example, after the refeeding of fifty-four *C. quinquefasciatus* dengue was not apparent until eleven and one-half to thirteen days had elapsed; while after the refeeding of over one hundred twenty-five *A. ægypti* dengue occurred after seven to nine days. In this connection it is of interest to note that three volunteers, inoculated with Mandler or Berkefeld filtrates of suspensions of infected mosquitoes, also had rather long incubation periods, which were believed to have resulted from the small amounts of virus injected. Whatever the real explanation may be, the experimental results indicate that relatively large numbers of mosquitoes are required for mechanical transmission of dengue or that it is rarely transmitted in this manner by individual mosquitoes. As it seems probable that such favorable conditions rarely occur naturally, it may be assumed that mechanical transmission of dengue by "interrupted feeding" of *C. quinquefasciatus* or *A. ægypti* can occur, but that it is probably of slight importance in the natural dissemination of dengue.

SUMMARY AND CONCLUSIONS

1. The virus of dengue was transferred mechanically by (*a*) fifty-four *Culex quinquefasciatus* and by (*b*) one hundred twenty-five *Aëdes ægypti,* which were interrupted while feeding on infective dengue blood and immediately allowed to complete feeding on susceptible volunteers.
2. The periods of incubation before dengue was recognized in these two volunteers were eleven and a half to thirteen days in one case and seven to nine days in the other.
3. Three similar transmission experiments in which only eight, twelve, and twenty-one *C. quinquefasciatus* were used resulted negatively.
4. These experiments indicate that relatively large numbers of mosquitoes are necessary for the transmission of dengue in this manner, and for this reason it is considered probable that such mechanical transmission rarely occurs in nature.

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OBSERVATIONS TO DETERMINE WHETHER OR NOT DENGUE VIRUS CAN PENETRATE NORMAL HUMAN SKIN

While the usual method of transmission of dengue by certain insect vectors is now well recognized, it was considered of some practical interest to determine whether or not the virus of dengue, like certain other viruses and bacteria, is able to penetrate normal human skin and in this way cause infection.

Such direct passage of the virus if it occurred would, of course, make it possible to contract dengue by contamination of the skin with blood or other virus-containing materials from dengue patients, with the crushed bodies of dengue-infected insects, or with the infective excretions of insects. The chronic and prolonged carrier state developed in *Aëdes ægypti* after infection with dengue virus allows one at least to speculate concerning the possibility that other species of blood-sucking insects, after feeding on dengue blood, might also become infected, even though anatomical or biological differences interfered with their transmission of the infection to man again during feeding. If such chronic dengue infections should occur in other insects, the possibility of transferring the virus to man by crushing them on the skin or by contamination of the skin with their excretions becomes a problem of practical importance.

In this connection the negative experiments of Ashburn and Craig,(1) who in 1907 attempted to infect eight healthy men by contact with dengue patients and constant exposure to fomites, are of considerable interest. They concluded that dengue is not a contagious disease. Cleland, Bradley, and McDonald(2) (1919) failed to cause dengue by application of infective serum to the scarified skin of a volunteer or by the application of infective materials to the nasal mucous membranes. However, they obtained a doubtful result when infective material was used as a throat gargle and then swallowed.

While these observations indicate that the virus of dengue as it exists in infective blood is probably not able to penetrate even the abraded human skin, recent reports concerning the penetration of the skin of monkeys by yellow-fever virus from infective mosquitoes suggested the advisability of further work with dengue along similar lines.

In 1928, it was observed by Bauer and Hudson(3) that the virus of yellow fever, which in many ways behaves similarly to dengue virus, when present in sufficient amount in the blood of animals can penetrate the intact skin of rhesus monkeys and produce infection. These workers were also able to infect a monkey with yellow fever by applying to the scarified skin one drop of a suspension prepared by macerating ten infected *Aëdes ægypti* in a mortar with 1 cubic centimeter of salt solution. However, similar amounts of this same suspension rubbed on the shaven skin of a second monkey and on the unbroken skin of another failed to produce the disease in both tests.

Marchoux(4) (1929) observed that members of the French Yellow Fever Commission in Brazil, during 1903, performed about one hundred autopsies with their bare hands, yet none of them became infected. However, as the virus practically always disappears from the blood and organs before death, this does not prove that yellow-fever virus cannot penetrate the skin. He also reported experiments in which applications of infective defibrinated blood to the conjunctiva and skin of animals failed to produce the disease; while emulsions of infected liver instilled into the conjunctival sac or placed on the excoriated skin did cause yellow fever.

Aragao and Lima(5) (1929) reported that yellow-fever virus in the fæces of infected *Aëdes ægypti*, when applied either to the intact conjunctiva or the unbroken skin of susceptible monkeys, caused the disease.

EXPERIMENTAL

In order to determine whether or not penetration of the skin by dengue virus is possible, and if so whether this might be of practical importance in the natural transmission of the disease, the following experiments were performed in which materials from mosquitoes infected with dengue were rubbed on the skin of susceptible human volunteers.

Experiment 1.—July 11, 1929. Two *A. ægypti* of lot 25, which lot had been proved to be infected with dengue, were stunned by jarring, and were immediately crushed on the inner portion of the middle third of the right arm of a susceptible human volunteer (A.V. 26–T). By means of the rounded end of a glass test tube the crushed mosquito bodies were then rubbed thoroughly over the intact skin. The volunteer, who had already been observed in a screened cubicle during a preliminary observation period of eleven days, did not develop dengue from this experiment. The volunteer's susceptibility to dengue was demonstrated twenty-three days later, when the feeding on him of eight dengue-infected *A. albopictus* of lot 29, July 29, resulted in dengue August 4. Comment: The crushed bodies of two presumably dengue-infected *A. ægypti* rubbed into the intact skin of a susceptible human volunteer failed to produce dengue in this volunteer.

Experiment 2.—July 11, 1929. Two mosquitoes from a dengue-infected lot of *A. ægypti* (lot 25) were killed and crushed in a similar manner on the skin of a susceptible human volunteer (A.V. 27–P). After the mosquitoes had been well rubbed in, the skin was abraded by the volunteer, who had been instructed to scratch the area as though it itched. As a result, serum and a trace of blood exuded from the scratches. The volunteer, who had had the usual preliminary observation period before the test, did not develop dengue from this procedure. His susceptibility was proved one month later when he developed dengue August 11, five days after being bitten by one infected *A. albopictus* of lot 29. Comment: Dengue virus from the crushed bodies of two presumably dengue-infected *A. ægypti*, scratched and rubbed into the abraded skin of a susceptible human volunteer, failed to cause dengue.

Experiment 3.—October 26, 1929. Eleven mosquitoes from dengue-infected *A. ægypti* lot 71 were allowed to feed on a volunteer (A.V. 55–S). After an incubation period of five days, dengue fever developed, proving that this portion of lot 71 contained infected mosquitoes. October 28, 1929, or two days after feeding on volunteer 55–S, all of these eleven mosquitoes were killed, ground with pyrex glass "sand" in a sterile porcelain mortar, and suspended in 1 cubic centimeter of physiologic salt solution. This saline suspension of mosquitoes and dengue virus was immediately applied to the scarified skin of a human volunteer (A.V. 56–K) in the following manner. With a sterile needle three linear scratches, each about 2 centimeters in length, were made in the thin skin over the upper anterior aspect of the forearm. While no blood was drawn, small amounts of serum appeared in the abrasions. Then about 0.2 cubic centimeter of the saline suspension of dengue virus was rubbed into each of two of the abrasions, leaving one uninoculated for control observations on any local reaction that might have developed. There was no local reaction nor did this procedure cause dengue. The volunteer was proved susceptible to the disease twenty-six days later when, November 23, 1929, dengue resulted from twenty-five *A. ægypti* of lot 104 which had fed on him seven days earlier. Comment: Four-tenths (0.4) of a cubic centimeter of a saline suspension representing approximately four dengue-infected *A. ægypti* rubbed into the abraded skin of a human volunteer failed to cause dengue.

DISCUSSION

Ashburn and Craig(1) in 1907 exposed healthy men to the fomites of dengue patients in a manner similar to the experiments of Walter Reed and his coworkers with yellow fever. Since eight volunteers exposed in this way failed to develop dengue, it was concluded that dengue could not be transferred by contact with patients. Cleland, Bradley, and McDonald(2) (1919) failed to transmit dengue when infective material was applied to the nasal mucous membranes or when dengue-infective serum was applied to the scarified skin.

The negative results obtained in our experiments do not, of course, prove that dengue virus is unable to penetrate the skin under all conditions. For example, the concentration of virus in the materials used may be an important factor, as was suggested by the work of Bauer and Hudson(3) with yellow-fever virus. These investigators observed that when blood drawn from animals at the beginning of yellow fever was applied to the unbroken skin of monkeys, infection resulted; while applications of blood that had been drawn later in the disease and, therefore, presumably contained less virus failed. Furthermore, when saline suspensions of infected mosquitoes were used yellow fever occurred only when the material was applied to abraded skin.

However, in the present work with dengue, it may be presumed that the materials used contained sufficient virus to cause infection if penetration of the skin had occurred, since two mosquitoes from a known infected lot were used in both experiments 1 and 2, while the saline suspension used in experiment 3 represented the equivalent of at least four and four-tenths mosquitoes known to have been infected.

CONCLUSIONS

1. In these experiments dengue virus from infected *Aëdes ægypti,* rubbed on both the intact and the excoriated skin of susceptible human volunteers, failed to penetrate the skin and cause dengue. Therefore, it seems that contamination of the skin by dengue virus from crushed *A. ægypti* or from other insects in which the virus might exist, is probably not a factor in the natural spread of dengue.

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OBSERVATIONS ON THE POSSIBILITY OF HEREDITARY TRANSMISSION OF DENGUE FROM INFECTED FEMALE AËDES ÆGYPTI THROUGH THE EGG TO THE OFFSPRING

The possibility of the transmission of dengue virus through the eggs of infected female *Aëdes ægypti* to their offspring, as the organism of Rocky Mountain spotted fever may be passed through the eggs of its vector, *Dermacentor andersoni,* was reconsidered, since such hereditary transfer, if proved, should be of great importance to those interested in the control of dengue.

In 1905, Marchoux and Simond,(1) in an attempt to find an explanation for the reappearance of yellow fever in places from which the disease had apparently disappeared, conducted experiments to determine whether or not this might be caused by mosquitoes that had inherited the infection by passage of the virus of yellow fever through the egg. According to their report they succeeded, in one instance, in producing yellow fever with mosquitoes that had hatched from the eggs of infected mosquitoes. However, Phillips(2) injected two hundred eggs laid by yellow-fever-infected mosquitoes into six normal *Macacus rhesus* monkeys, with negative results. In five biting and two injection experiments with progeny of the same infective lot of mosquitoes, using seven normal monkeys, the results were also negative. Consequently, it was concluded that under the conditions of his experiments hereditary transmission of yellow fever by *A. ægypti* was improbable.

Siler, Hall, and Hitchens(3) investigated this possibility with mosquitoes infected with dengue. They collected the eggs laid by *A. ægypti* that had been proved to be infected with dengue virus; and fed the adults raised from these eggs on sugar solutions for periods of three to forty-two days, after which they were allowed to feed on three human volunteers. Eight days later the various lots were again applied to the same volunteers with negative results. After another period of eight days the volunteers were tested to determine their susceptibility by exposure to lots of infective *A. ægypti.* It was shown that one of the volunteers was immune to dengue, but two were susceptible. The following statement was made concerning the results: "We believe the conclusion is justified that the virus of dengue fever does not pass from the infected *Aëdes* through its eggs to the next succeeding generation."

EXPERIMENTAL

It was considered possible that even if dengue virus should pass from the infected female through the egg to the offspring, the latter might not be able to transmit the infection to man until twelve or more days had elapsed following the first feeding of normal blood. With this possibility in mind, the following experiment was performed.

Experiment 1.—December 15, 1928, fifty normal *Aëdes ægypti* took full feedings of infective blood from a case of dengue (Keller) during the second day of the disease and were designated lot 1. This was the first passage of the strain of dengue virus used throughout the various investigations made by the present board. Lot 1 *A. ægypti* transmitted dengue by feeding December 31, 1928, on two susceptible volunteers (A.V. 1–H and A.V. 2–M). Between January 4 and 8, 1929, or from twenty to twenty-four days after the infective feeding on Keller, the mosquitoes of lot 1 laid eggs which were collected and placed in a jar of water. Between January 15 and 20, 1929, the adult mosquitoes emerged and were fed on the blood of a normal guinea pig as follows: January 21, twelve fed; January 22, nineteen fed; January 23, eighteen fed, and January 24, eleven fed; making a total of sixty mosquitoes, which were combined and designated *A. ægypti* lot 8.

February 12, or from nineteen to twenty-two days after feeding on normal guinea-pig blood, forty-eight mosquitoes of lot 8 took full feedings of blood from a susceptible volunteer (A.V. 4–Y). Dengue did not develop during an observation period of thirteen days. However, after being tested for susceptibility with a known infective lot of *A. ægypti* (No. 2) February 19, 1929, this volunteer had dengue fever seven and one-half days later, on the evening of February 26, 1929.

CONCLUSION

The negative result obtained in this experiment agrees with the observations reported by Siler, Hall, and Hitchens and indicates that dengue virus is not transmitted from infected female *A. ægypti* through the egg to the offspring.

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EXPERIMENTAL ATTEMPTS TO TRANSMIT DENGUE VIRUS DIRECTLY TO LARVÆ OF NORMAL AËDES ÆGYPTI

The extreme susceptibility of adult *Aëdes ægypti* to infection with dengue virus, and the long chronic carrier state that follows infection of this mosquito, suggested the possibility that *A. ægypti* might also be infected during the larval stage by ingestion of dengue virus, added intentionally or accidentally to the fluids in which they are developing, and that if such infection were acquired it might persist throughout the adult stage of the mosquito. If it were possible to infect *A. ægypti* larvæ in such a manner, the question would, of course, arise as to the possibility of transferring dengue virus in nature directly from infected adult mosquitoes to normal larvæ by the accidental contamination of small breeding places, with the infective secretions or the dead bodies of the former. Such an occurrence would indicate the possibility of maintaining the virus of dengue in endemic localities by mosquitoes alone, without the necessity of alternate passages through vertebrate hosts.

No reference was found in the available literature to such investigations with dengue. However, according to Wittingham,(1) in 1924 the Royal Air Force Sandfly Commission observed that larvæ of *Phlebotomus* which were obtained from adults infected with the virus of sand-fly fever and bred under sterile conditions, except that they may have ingested the dejecta or dead remains of the parent flies, became infected after a preliminary feeding of normal human blood, and the adults that developed from these larvæ were able to transmit the disease to man.

EXPERIMENTAL

In these studies, eggs from normal *Aëdes ægypti* were placed in fruit jars containing 100 to 500 cubic centimeters of tap water. After the eggs had hatched, thin slices of ripe banana were added to each jar as food for the larvæ. At various intervals thereafter, suspensions of adult *A. ægypti* infected with dengue virus were added. The adult mosquitoes that developed from these larvæ were later tested for infectivity by feeding on susceptible human volunteers.

Experiment 1.—August 9, 1929. About one hundred recently hatched *A. ægypti* larvæ were placed in a fruit jar containing 500 cubic centimeters of tap water and a slice of banana. To this water was added a suspension prepared by macerating in distilled water five dengue-infected *A. ægypti* of lot 32. Similar suspensions, each made with five infective mosquitoes, were prepared daily and added to the water August 10, 11, 12, 13, 14, 15, and 16. August 17 the water in the jar was found to be slightly acid to litmus and was changed in the following manner. The larvæ were transferred to a clean jar containing 50 cubic centimeters of fresh tap water to which a suspension of five dengue-infected adult mosquitoes had been added. One hour later 400 cubic centimeters of clean water was added and the jar was placed in a screened cage. As some of the mosquitoes were already in the pupal stage, a few adults emerged on the following day, August 18. By August 20 only a few larvæ remained in the jar and the cage contained a considerable number of adult male and female mosquitoes. The females were separated and designated *A. ægypti* lot 62–B.

Test for infectivity.—August 26, 1929, or at least six days after they had emerged, thirty-three female mosquitoes of lot 62–B took blood from a susceptible volunteer (A.V. 36–V). Eight days later (September 3) some of the mosquitoes fed again on the same volunteer, and a third feeding with lot 62–B took place September 11, 1929. Dengue did not develop between September 11 and 26. The susceptibility of this volunteer was proved by another experiment in which twenty-eight *A. ægypti* of combined lots 44, 45, 46, and 47, which fed September 21, produced dengue after an incubation period of five days on September 26, 1929. Comment: In this experiment *A. ægypti* that had lived during their larval stage in 500 cubic centimeters of tap water containing banana, to which was added daily suspensions of five infected adult mosquitoes, failed to transmit dengue by feeding on a susceptible volunteer.

Experiment 2.—November 18, 1929. About two hundred recently hatched normal *A. ægypti* larvæ were placed in each of three fruit jars containing 250 cubic centimeters of distilled water, to which were added two macerated *A. ægypti* of lot 101 which had taken infective blood from A.V. 55–S during the first day of dengue. The infectivity of lot 101 was never determined, but the presence of dengue virus in the blood of A.V 55–S at the time of blood feeding was proved by lot 104, which later caused dengue in A.V. 56–K and A.V. 49–L. Subsequently, *A. ægypti* of lot 101 were added to the water in each jar as follows: November 19, two mosquitoes were added. November 20, two mosquitoes, were added; at 9 a. m., and at 12 noon a small slice of banana was placed in each jar. November 21, at 9 a. m., larvæ were transferred to bottles, each of which contained 30 cubic centimeters of distilled water and two mosquitoes of lot 101; and were left in the bottles for three hours, after which they were returned to the jars with 250 cubic centimeters of water. November 22, 23, and 24, two mosquitoes of lot 101 were added daily to each jar. November 25, at 9 a. m., larvæ were transferred to bottles containing 30 cubic centimeters of distilled water and two mosquitoes of lot 101, and were left in these bottles until the following day. November 26, at 10 a. m., the larvæ were returned to the jars and ten mosquitoes of lot 101, which had died during the previous day, were added to each jar; at noon thin slices of banana were added. November 27 and 28, two mosquitoes of lot 101 were added. November 29, at 7 a. m., the larvæ were transferred to bottles containing 30 cubic centimeters of distilled water and two mosquitoes of lot 101 and at noon they were returned to the jars containing 250 cubic centimeters of water and slices of banana. November 30 and December 1 and 2, two mosquitoes of lot 101 were added daily to each jar. December 3, at 8 a. m., larvæ were transferred to bottles containing 30 cubic centimeters of distilled water and two mosquitoes of lot 101 and were left until noon, after which they were returned to the jars as usual. December 4 and 5, two mosquitoes of lot 101 were added daily to each jar. December 6, larvæ were transferred to new jars with fresh water and banana. December 8, larvæ were transferred to clean fruit jars containing fresh water and banana and ten mosquitoes of lot 101 were added to each. The first pupæ appeared November 30, and as they developed they were transferred into bottles of distilled water and adults were allowed to emerge in a screened cage labeled "*A. ægypti* lot 127."

Test to determine infectivity of A. ægypti lot 127.—December 19, 1929, eighty-five mosquitoes of lot 127 took feedings of normal blood from Staff Sergeant Rhodes. After an interval of thirty-four days, one hundred ten mosquitoes of lot 127 fed on a volunteer (A.V. 76–M) at 8 a. m., January 22, 1930. Dengue did not develop during sixteen days observation. The susceptibility of this man (A.V. 76–M) to dengue was proved by the fact that sixty-one infective *A. ægypti* of lot 137, which fed on him February 1, 1930, produced dengue February 7, 1930. Comment: In this experiment *A. ægypti*, which, during the larval stage had developed in water repeatedly contaminated by additions of macerated potentially infected *A. ægypti*, failed to produce dengue in a susceptible human volunteer.

DISCUSSION

While the failure to infect *Aëdes ægypti* larvæ with dengue in these experiments seems to indicate that such infection may not be of importance in the natural spread of the virus, it is, of course, possible that larvæ might be infected under slightly different circumstances. So little is known concerning the biology of the virus of dengue, its viability in various mediums and under different conditions of temperature and hydrogen-ion concentration, or concerning the amounts of virus present in mosquitoes or other infective materials, that it will be necessary to investigate the question further before it can be stated that larvæ of *A. ægypti* are not susceptible to infection. For instance, it seems possible that after suspensions of infected mosquitoes were added to the water, the dilution of virus might have been so great that none of it was ingested by the larvæ; it is also possible that the virus was unable to survive in the water long enough to reach the larvæ in an infective condition. Under natural conditions *Aëdes* larvæ may be found developing in small collections of water in receptacles containing many different substances, which no doubt influence its hydrogen-ion reaction and its nutritive value, and it seems possible that further investigation might show that larvæ may be infected with dengue by the ingestion of virus contaminating the medium in which they develop.

SUMMARY AND CONCLUSIONS

1. Adult female *Aëdes ægypti*, developed from larvæ that had lived in water contaminated at intervals by the addition of small amounts of macerated infective adult mosquitoes, failed to transmit dengue to susceptible human individuals.
2. However, it was not possible to be certain that the dengue virus added to the water was ingested by these larvæ or that it remained alive long enough to cause infection even if it were ingested.
3. Further investigation may show that under different conditions mosquito larvæ may be infected with dengue virus.

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OBSERVATIONS ON THE POSSIBILITY OF TRANSMITTING DENGUE VIRUS FROM INFECTED TO NORMAL AËDES ÆGYPTI THROUGH CONTAMINATED FOOD OR WATER

As dengue virus escapes from the proboscis of infective female *Aëdes ægypti* while they are feeding on blood, it was considered possible that a similar escape of the virus might occur during the ingestion of other foods or water, and make it possible for normal mosquitoes feeding subsequently on the "contaminated" material to ingest the virus and become infected.

EXPERIMENTAL

In the following experiments an attempt was made to transmit dengue in this manner, by placing banana, papaya, sugar solutions, and water in the cages of infected mosquitoes for various periods of time and then transferring these materials to cages containing normal mosquitoes. After intervals of several days, the latter groups of mosquitoes were tested for infectivity by allowing them to feed on susceptible human volunteers.

Experiment 1.—August 1, 1929. Small glass containers for banana and papaya were prepared from sections of glass tubing, and cups for water were made by cutting off the lower portion of homeopathic vials. A fresh set of these containers, filled with water and fruit, respectively, was placed each day in a cage containing a large number of infective *A. ægypti*, lot 32. After use for twenty-four hours, the food and water containers were transferred to a cage containing two hundred normal *A. ægypti.* Large numbers of both groups of mosquitoes fed on the same materials. This procedure was kept up daily for several weeks, after which the normal-group mosquitoes were allowed to feed on normal human blood and reserved for later tests on human volunteers. Unfortunately, the mortality was so great among these mosquitoes that their infectivity could not be determined.

Experiment 2.—August 14, 1929. Papaya, banana, and water were transferred daily, as in experiment 1, from a cage containing infective *A. ægypti*, lot 30, to a cage containing one hundred normal *A. ægypti*, which were called lot 62–A. September 14, or thirty-one days after the beginning of the experiment, the six mosquitoes remaining in lot 62 were given an opportunity to feed on a susceptible volunteer (A.V. 46–L). Four of the mosquitoes took blood, but dengue did not develop during an observation period of twenty-three days. Comment: Because of the fact that only four of the original one hundred mosquitoes were available for test on the volunteer, these negative results were considered to be inconclusive.

Experiment 3.—November 18, 1929. A clean sterile Petri plate, containing approximately 15 cubic centimeters of 5 per cent sugar solution in tap water, was placed in a cage containing one hundred female *A. ægypti* selected from lot 101 and designated lot 101–A. The infectivity of these mosquitoes was indicated by the fact that other *A. ægypti*, lot 104, which fed at the same time as lot 101, transmitted dengue by feeding on human volunteers (A.V. 56–K and A.V. 49–L). After twenty-four hours (November 19) the sugar solution was transferred from the cage of *A. ægypti*, lot 101–A, to a cage containing one hundred normal *A. ægypti*, which were designated lot 121. Another clean dish of fresh sugar solution was given to lot 101–A. This routine transfer of used sugar solution from lot 101–A to lot 121 was continued daily for fifteen days, between November 20 and December 5, at which time only sixteen mosquitoes of lot 101–A remained alive.

TESTS FOR INFECTIVITY

Test (*a*).—November 28, 1929, 8.15 a. m. Susceptible volunteer A.V. 59–W was bitten by sixty-three of the sixty-seven mosquitoes remaining in lot 121 but dengue did not result.

Test (*b*).—December 5, 1929, 11.30 a. m. Forty-five mosquitoes of lot 121 again fed on the volunteer (A.V. 59–W) and again failed to cause dengue.

Test (*c*).—December 12, 1929. Thirty-one mosquitoes of lot 121 fed a third time on A.V. 59–W with similar negative results.

Test (*d*).—January 14, 1930, 1 p. m. Sixteen *A. ægypti* of lot 121 fed on another susceptible volunteer (A.V. 74–L), who also failed to develop dengue during an observation period of seventeen days.

Comment.—Normal *A. ægypti* of lot 121, which had fed daily for sixteen days on 15 cubic centimeter amounts of 5 per cent sugar solution that had been used by infective mosquitoes for twenty-four hours previously, were tested four times and failed to transmit dengue to man.

Experiment 4.—November 18, 1929. A sterile Petri plate containing fresh tap water and another plate containing a thin slice of ripe banana were placed in a cage containing one hundred eleven infective female *A. ægypti* selected form lot 101 and designated lot 101-B. Twenty-four hours later (November 19) the water and banana were replaced by fresh materials in new containers, and the previously contaminated water and food were transferred to a cage containing one hundred normal female *A. ægypti*, which were designated lot 122. Similar transfers of food and water were made daily for sixteen days, between November 19 and December 5, 1929.

TESTS FOR INFECTIVITY OF AËDES ÆGYPTI, LOT 122

Test (*a*).—November 28, 1929, 8.15 a. m. Sixty of the sixty-eight remaining mosquitoes of lot 122 fed on a susceptible volunteer (A.V. 59–W) but failed to produce dengue.

Test (*b*).—December 5, 1929, 11.30 a. m. Nineteen mosquitoes of lot 122 again fed on the same volunteer (A.V. 59–W) with similar negative results.

Test (*c*).—December 12, 1929, 9 a. m. Twenty-six mosquitoes of lot 122 fed on A.V. 59–W and failed to cause dengue.

Test (*d*).—January 14, 1930, 1 p. m. Six mosquitoes of lot 122 fed on a susceptible volunteer (A.V. 74–L) and failed to produce dengue.

Comment.—Normal *A. ægypti* of lot 122, fed daily for sixteen days on banana and water that had been used by infective mosquitoes for twenty-four hours previously, were tested four times and failed to transmit dengue to man.

Experiment 5.—March 10, 1930. One hundred thirty normal female *A. albopictus*, later called lot 182, were liberated in a cage with one hundred infective female *A. ægypti* of lot 168, which had fed on the blood of a dengue patient twenty-one days previously and had transmitted dengue to two human volunteers (A.V. 83–R and A.V. 87–R). All mosquitoes of both lots, No. 186 (*A. albopictus*) and No. 168 (*A. ægypti*), were inspected individually and identified before they were placed together in the cage. A small glass dish containing 15 cubic centimeters of a 5 per cent sugar solution was then added, in order that the normal *A. albopictus* might feed on the same solution as the infective *A. ægypti*. The food material was renewed daily during eleven days of the experiment.

TESTS TO DETERMINE INFECTIVITY OF AËDES ALBOPICTUS, LOT 182

Test (*a*).—March 22, 1930, 11 a. m. Sixty-eight of the remaining *A. albopictus*, lot 182, collected from the two mixed lots, fed on a susceptible volunteer (A.V. 92–C), but failed to produce dengue.

Test (*b*).—March 31, 1930, 10.30 a. m. Twenty-three *A. albopictus* of lot 182 again fed on the same volunteer (A.V. 92–C) and as dengue did not develop during an observation period of ten days, the susceptibility of this volunteer was tested April 10 at 12.30 p. m., by feeding on him seventy-three infective *A. ægypti* of lot 189. Two days five hours after this feeding dengue began in the afternoon of April 12. As this incubation period is shorter than any previously observed, it seems possible that the lot 182 *A. albopictus* might have caused the disease after a rather long incubation period of twelve days seven hours.

Comment.—From this experiment no final conclusion can be drawn concerning the possibility of transmitting dengue from infected *A. ægypti* to normal *A. albopictus* through contaminated food. However, the findings are sufficiently suggestive to warrant further investigation.

SUMMARY AND CONCLUSIONS

Five attempts were made to infect normal *Aëdes* by feeding them on food materials or water that had been used during the previous day by infective mosquitoes. In one experiment the mosquitoes died before they could be tested on a susceptible human volunteer; in one experiment only four mosquitoes remained for the test, which was negative; in two definitely negative experiments the mosquitoes failed to infect susceptible volunteers; and in one experiment in which normal *A. albopictus* were placed in the same cage with infected *A ægypti,* the results were suggestive but inconclusive. Thus, no positive evidence was obtained in these experiments to indicate that such transmission may occur. However, in the light of other experiments in which normal *Aëdes* were infected by the ingestion of macerated infected mosquitoes suspended in normal blood, it is believed possible that under favorable conditions mosquitoes may become infected with dengue virus in nature through the agency of contaminated food or water.

TRANSMISSION OF DENGUE VIRUS BY FEEDING NORMAL AËDES ÆGYPTI ON SUSPENSIONS OF INFECTED MOSQUITOES

In the following experiments dengue virus was transmitted directly from infective to normal *Aëdes ægypti* without an intermediate passage through a warm-blooded host, by macerating and suspending the infective mosquitoes in normal blood and feeding this suspension to the normal mosquitoes. This was made possible by the development of a satisfactory method (1) for feeding mosquitoes artificially on blood and other materials, which is described below.

EXPERIMENTAL

Technic.—The fresh, clean skin of a young, normal, exsanguinated guinea pig or chicken, freed of subcutaneous fat and hair or feathers, was clamped tightly over the shallow depression on the under surface of the lid of an ordinary staining dish (Plate 2, fig. 1) by means of a wire frame held in place by rubber bands or by sealing with paraffin. The air was removed from the cell with a Luer syringe and replaced by a mixture of citrated or defibrinated normal blood[4] and macerated dengue-infected *Aëdes ægypti.* Sufficient material was injected to cause a distinct bulging of the skin, after which the needle hole was sealed with melted paraffin. The mixture was kept at approximately 37° C. by placing the apparatus over a glass dish half filled with water heated to 55° C. The entire apparatus was then inserted into a cage containing normal laboratory-bred *A. ægypti.* The mosquitoes were at once attracted to the skin, and in a short time most of them became engorged with the blood mixture (Plate 2, fig. 2). This technic was carried out on eight different occasions with successful results in four.

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[4] The blood used was obtained from volunteers during the observation period preliminary to dengue experimentation and in one instance from a normal guinea pig. The human blood used was proved to be free from the virus at the time it was taken, by subsequent observation of the volunteers for eight days, and by producing dengue fever in these individuals, a few days after the end of the observation period, with known infected mosquitoes.

Experiment 1.—August 20, 1929. Eighty infective *Aëdes ægypti* of lot 32, which had fed thirty-four days previously on an experimental case of dengue (A.V. 21–M) during the first day of the disease, and had been proved infected by transmitting dengue fever to A.V. 28–P and A.V. 29–J, were macerated in a mortar containing sterile sand, 1 cubic centimeter of 0.85 per cent salt solution (NaCl), and 4 cubic centimeters of normal human blood and fed to twenty-two normal *A. ægypti*, which were designated lot 44.

August 20, 1929. Twenty-five infective *A. ægypti* of lot 35, which had fed sixteen days previously on an experimental case of dengue (A.V. 28–P), during the first day of the disease, and had been proved infective by transmitting dengue fever to A.V. 31–D, were macerated with sand and 4 cubic centimeters of citrated human blood; and fed to twenty-two normal *A. ægypti*, which were designated lot 45.

August 22, 1929. Forty *A. ægypti* of lot 40, which had fed seventeen days previously on an experimental case (A.V. 20–B), at the beginning of the second day of dengue fever, but had not subsequently been proved infective, were macerated and suspended in 3 cubic centimeters of guinea-pig blood, and fed to seven normal *A. ægypti*, which were designated lot 46.

August 23, 1929. Thirty infective *A. ægypti* from lot 40, and thirty from lot 41, which had fed eighteen and seventeen days previously on experimental cases A.V. 20–B and A.V. 23–B, were macerated and suspended in 5 cubic centimeters of normal human blood and fed to one hundred one normal *A. ægypti*, which became lot 47.

September 5, 1929. Thirteen to sixteen days after the mosquitoes of lots 44, 45, 46, and 47 had ingested blood from the dengue patients, they were tested for infectivity by allowing forty-seven of them to feed on a susceptible volunteer (A.V. 39–M); who, after an incubation period of six days, developed a typical unmodified case of dengue fever, which continued for seven days.

September 21, 1929. Twenty-nine to thirty-two days following the infective feedings by lots 44, 45, 46, and 47, twenty-eight fed on A.V. 36–V, who five days later developed a typical and rather severe case of dengue fever. The diagnosis in this case was further proved by the passage of the virus from this volunteer through *A. ægypti* lot 71 to A.V. 48–H and A.V. 55–S and subsequently through several human subjects and different lots of mosquitoes.

Conclusions.—Normal *A. ægypti*, which had fed on blood containing a suspension of macerated dengue-infected mosquitoes in the proportions of 1.6, 0.625, 1.33, and 1.20 mosquitoes to 0.1 cubic centimeter of fluid, became infected as was proved by the transmission of the disease to two susceptible American volunteers.

Experiment 2.—September 23, 1929. Five *A. ægypti*, of lot 48, which had fed twenty-seven days previously on A.V. 29–J during the first day of dengue fever, but which had not been tested, were macerated and suspended in 8.5 cubic centimeters of normal human blood, and fed to one

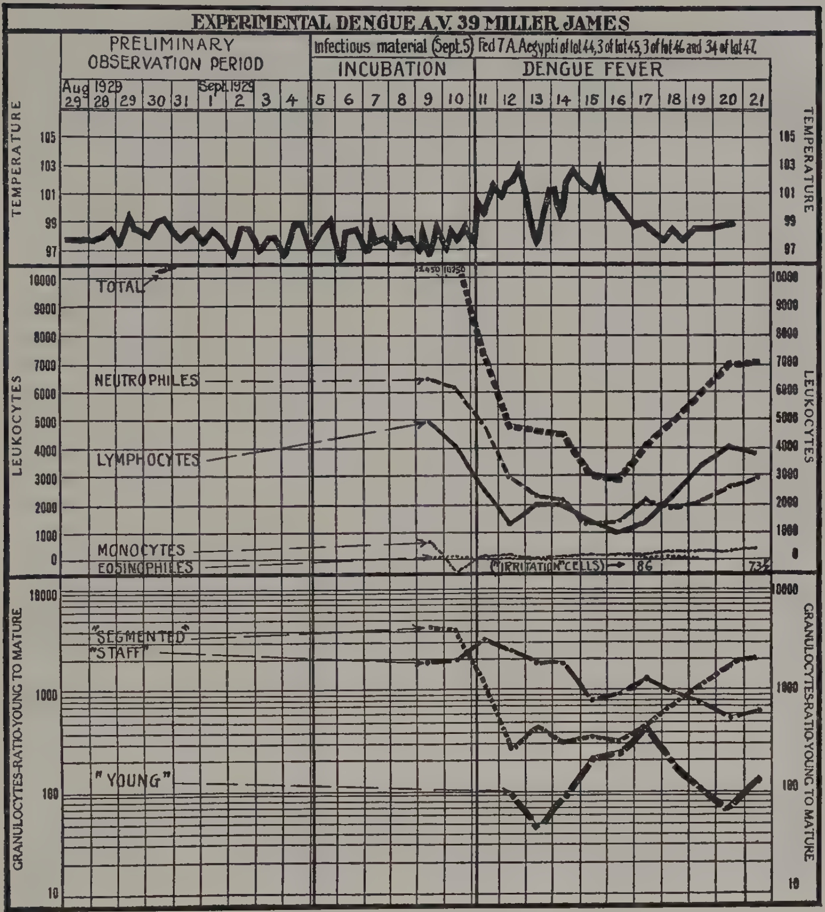

FIG. 12. Experimental dengue in American volunteer 39.

hundred forty-eight normal *A. ægypti*, which were designated lot 67 and tested for infectivity as follows: October 7, 1929, fourteen days following feeding on this mosquito suspension, six *A. ægypti* of lot 67 fed on a volunteer (A.V. 49–L), but no infection followed. Eight days later (October 15), or twenty-two days after the original feeding, forty-seven mosquitoes of lot 67 again fed on the same volunteer. Dengue did not follow during a prolonged observation period, and the volunteer was subsequently proved susceptible by the feeding of twenty-one infective *A. ægypti* of lot 104, November 16, 1929, which produced dengue November 21, 1929.

Conclusions.—The feeding of normal *A. ægypti* on a dilute suspension of presumably dengue-infected *A. ægypti* (0.058 mosquito per 0.1 cubic centimeter of blood) did not result in their infection as was indicated by the fact that small and large groups fed on a susceptible volunteer failed to transmit dengue.

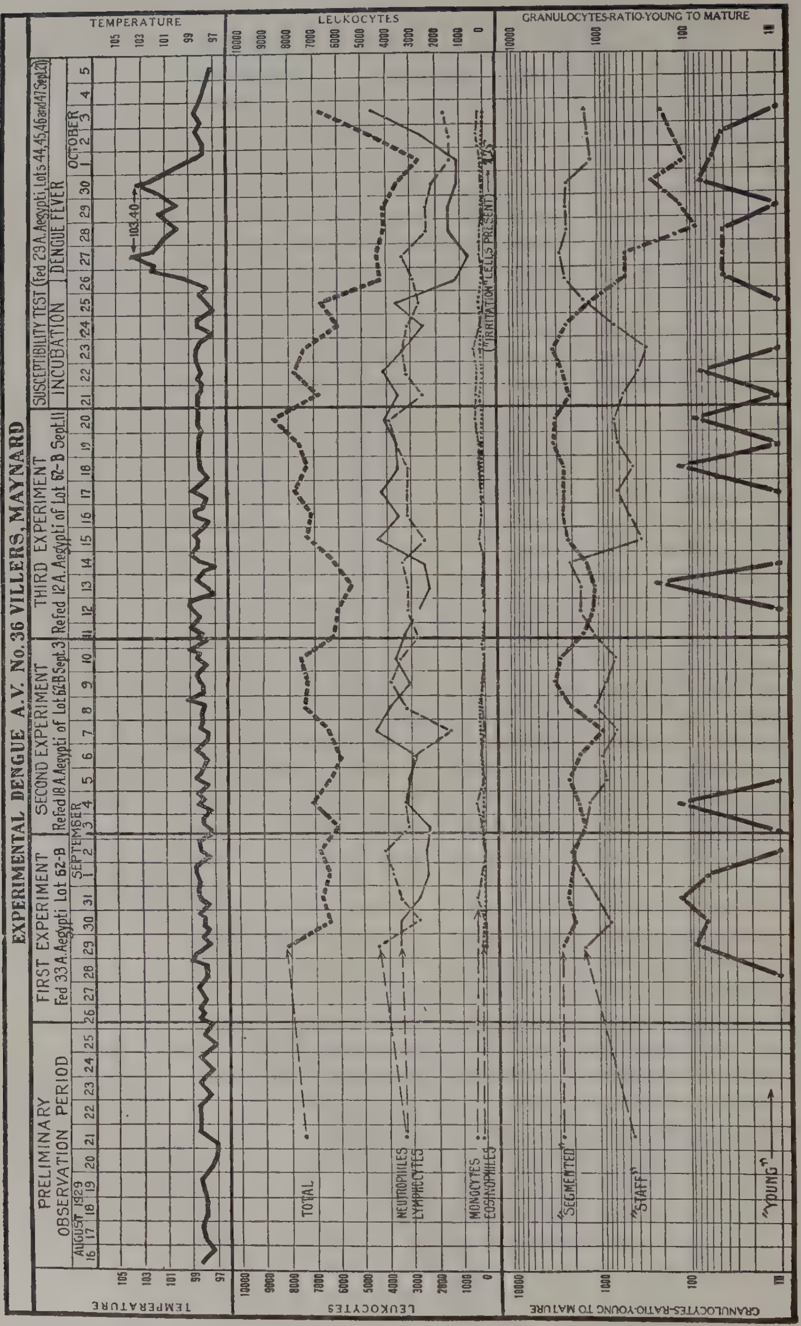

FIG. 13. Experimental dengue in American volunteer 36.

Experiment 3.—September 23, 1929. Fifty *A. ægypti* of lot 48, which had fed twenty-seven days previously on an experimental dengue case (A.V. 29–J) during the first day of fever, but had not been tested, were ground in a mortar containing a little sand and a few drops of hormone broth. After adding sufficient hormone broth to make a total volume of 10 cubic centimeters, an attempt was made to feed this material to normal *A. ægypti.* As the mosquitoes failed to feed, 5 cubic centimeters of the suspension was removed and replaced by 5 cubic centimeters of normal blood. Fifty-eight normal *A. ægypti* fed on this mixture and were designated lot 68. October 23, or thirty days later, seventeen mosquitoes of lot 68 were tested for infectivity by feeding on a volunteer (A.V. 49–L). Dengue did not follow during an observation period extending to November 16. The volunteer was subsequently proved susceptible by the infective feeding of *A. ægypti,* lot 104, November 16, 1929, which resulted in dengue November 21, 1929.

Conclusions.—Normal *A. ægypti* fed on a mixture of equal parts hormone broth and normal blood, which contained a suspension of presumably dengue-infected *A. ægypti* in the proportion of 0.25 mosquito to 0.1 cubic centimeter of fluid, failed to transmit dengue to man.

Experiment 4.—October 30, 1929. Eighty-one *A. ægypti;* namely, six of lot 52, eight of lot 54, seven of lot 59, one of lot 60, two of lot 61, five of lot 71, ten of lot 72, thirty-two of lot 76 and ten of lot 79, which had fed on the blood of experimental dengue patients from twenty-one to sixty days previously, were ground in a mortar containing 1 cubic centimeter of 0.85 per cent salt solution and 1 cubic centimeter of normal human blood. Thirty-three normal *A. ægypti* that fed on this mixture were designated lot 96.

November 11, 1929. Twelve days later the mosquitoes of lot 96 were tested for infectivity by feeding thirty of them on a volunteer (A.V. 57–E). This man developed dengue fever, which began five days later.

Conclusions.—Normal *A. ægypti* fed on blood containing dengue-infected mosquitoes (4.05 per 0.1 cubic centimeter of fluid) were infected as was proved by their transmission of dengue to a volunteer (A.V. 57–E) and the subsequent transfer of the infection from this man through lot 114 *A. ægypti* to another volunteer (A.V. 66–W).

Experiment 5.—March 5, 1930. One hundred *A. ægypti* of lot 165, which twenty-five days previously had fed on an experimental case (A.V. 76–M), during the first day of dengue fever, and had subsequently transmitted dengue to A.V. 86–B, were ground in a mortar containing powdered pyrex glass, 1 cubic centimeter of 0.85 per cent salt solution (NaCl) and 2 cubic centimeters of normal defibrinated human blood and fed to fifty-two normal *A. ægypti,* which were designated lot 177 and tested for infectivity as follows:

March 22, 1930. Seventeen days after feeding on the mosquito suspension sixteen mosquitoes of lot 177 were fed on a susceptible volunteer (A.V. 93–R). Dengue did not follow and the volunteer's susceptibility was proved subsequently by means of lot 183 *A. albopictus.*

Conclusions.—Normal *A. ægypti* fed on a suspension of infective mosquitoes (3.33 mosquitoes per 0.1 cubic centimeter of blood) failed to transmit dengue to man.

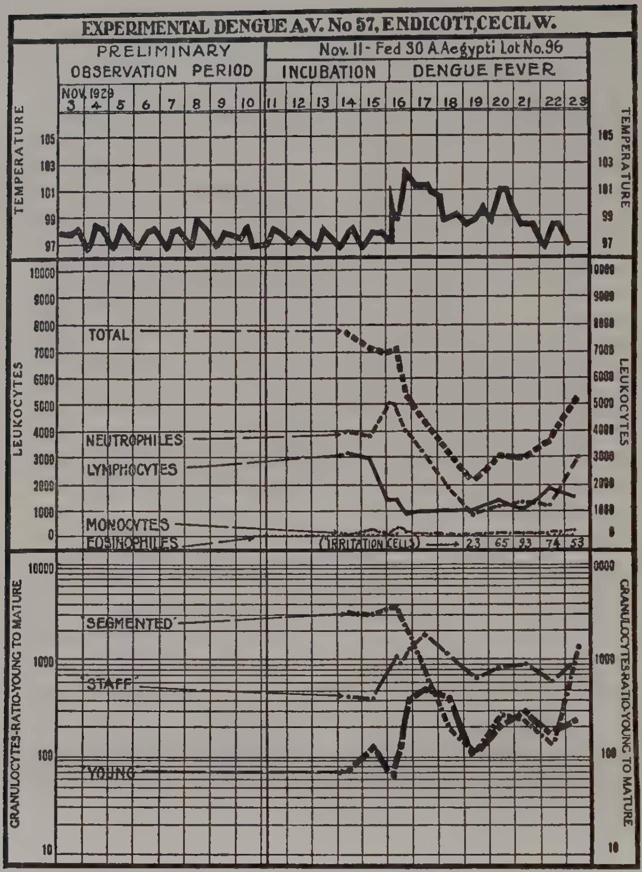

FIG. 14. Experimental dengue in American volunteer 57.

Experiment 6.—March 5 and 7, 1930. On these dates two groups, each composed of one hundred *A. ægypti* of lot 168, which had fed sixteen and eighteen days previously on an experimental dengue case (A.V. 78–O) during the first day of fever, and had transmitted dengue to two volunteers (A.V. 83–R and A.V. 87–R), were ground in a mortar containing powdered pyrex glass, 1 cubic centimeter of 0.85 per cent salt solution, and 2 cubic centimeters of normal human blood. This material was then fed to sixty-five and sixty-three *A. ægypti*, respectively, which were, designated lots 178 and 181, and later tested for infectivity as follows:

March 14, 1930. Nine and seven days, respectively, following the feeding of lots 178 and 181 on the mosquito suspension, forty-four and thirty-two of them fed on A.V. 88–B, but failed to cause dengue. However, when twenty-eight and nineteen of these mosquitoes were fed again, March

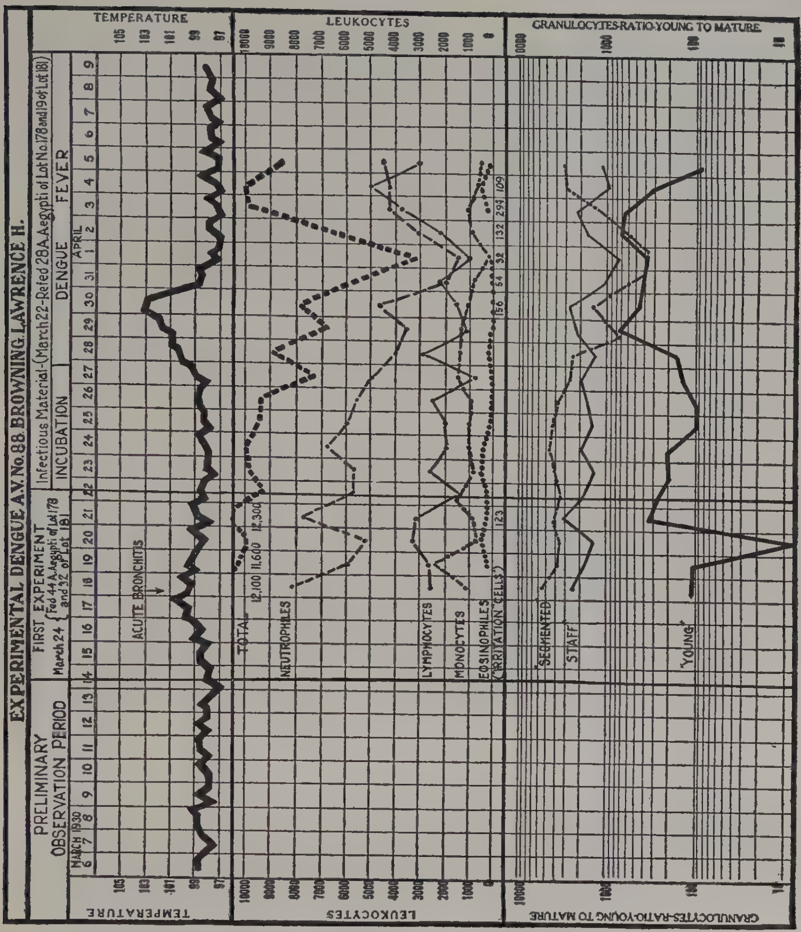

Fig. 15. Experimental dengue in American volunteer 88.

22, on the same volunteer a mild but typical case of dengue developed, after an incubation period of five days.

Comments.—(1) As dengue was not transmitted by lots 178 and 181 nine and seven days after they had ingested the suspension but was carried by these mosquitoes after the intervals had become seventeen and fifteen days, it appears that the former interval of time was too brief for the development of the virus within the mosquitoes.

(2) Normal *A. ægypti* were infected by feeding on a suspension of macerated dengue-infected *A. ægypti* containing 3.33 mosquitoes per 0.1 cubic centimeter of fluid.

Experiment 7.—September 16 and 17, 1929. On these dates attempts were made to effect a second serial transfer of the virus of dengue from mosquito to mosquito. September 16, eighteen *A. ægypti* of lots 44, 45, 46, and 47, which had previously been infected by feeding on suspensions of mosquitoes (see experiment 1, Table 6), were macerated in a mortar containing sand and 4 cubic centimeters of defibrinated normal human blood, and fed to thirteen normal *A. ægypti*. September 17, twenty-four additional mosquitoes of the same lots (44, 45, 46, and 47) were macerated and suspended in 5 cubic centimeters of normal human blood and fed to eleven normal *A. ægypti*. The mosquitoes were combined, designated lot 63, and tested for infectivity as follows:

October 2, 1929. Fifteen and sixteen days, respectively, after the feeding on the infective suspension, seven *A. ægypti* of lot 63, fed on a susceptible volunteer (A.V. 44–F); but dengue did not result. The susceptibility of this man was proved subsequently with lot 52 *Aëdes ægypti*.

Conclusion.—The attempt to transfer dengue virus in series by feeding normal *A. ægypti* on a normal blood suspension of mosquitoes (0.45 and 0.48 mosquito per 0.1 cubic centimeter), which themselves had been infected by feeding on a suspension of infective *A. ægypti*, was unsuccessful.

Experiment 8.—September 23, 1929. A second attempt was made on this date to transfer dengue virus in series from mosquito to mosquito. Thirty *A. ægypti* of the combined lots 44, 45, 46, and 47 (see experiment 1) were macerated and suspended in 1 cubic centimeter of normal human blood, and 5 cubic centimeters of 0.85 per cent salt solution. One hundred thirty-five normal *A. ægypti*, which fed on this suspension, were designated lot 69. September 28, or five days after this feeding, ninety-five mosquitoes of lot 69 were fed on a volunteer (A.V. 47–D); as dengue did not develop during the following eight days, fifty mosquitoes of the same lot were again fed on A.V. 47–D October 7. After an incubation period of five days a mild attack of dengue developed. The diagnosis was further confirmed by transfer of the virus from A.V. 47–D through *A. ægypti* lot 86 to two other volunteers, A.V. 51–S and A.V. 50–C, both of whom developed typical dengue.

November 4, 1929. Forty-two days after the infective feeding, ten mosquitoes of lot 69 were again tested on a volunteer (A.V. 56–K). As dengue did not develop during the following twelve days, the volunteer was tested for susceptibility November 16, 1929, by feeding *A. ægypti* of lot 104. A typical, moderately severe attack of dengue developed November 23, 1929.

Comments.—(1) Apparently infections occurred only in certain individual mosquitoes of the groups that fed on the suspension representing a dilution of 0.5 mosquito per 0.1 cubic centimeter of saline and blood, and these infected mosquitoes had died before the second test. The mosquitoes used in preparing this suspension had themselves been infected by feeding on a suspension of dengue mosquitoes; therefore, this experiment represented a second serial transfer of dengue virus from infected to normal mosquitoes.

(2) This experiment also demonstrates that the mosquitoes are not infective five days after feeding on other mosquitoes but are able to transmit the infection after an interval of fourteen days.

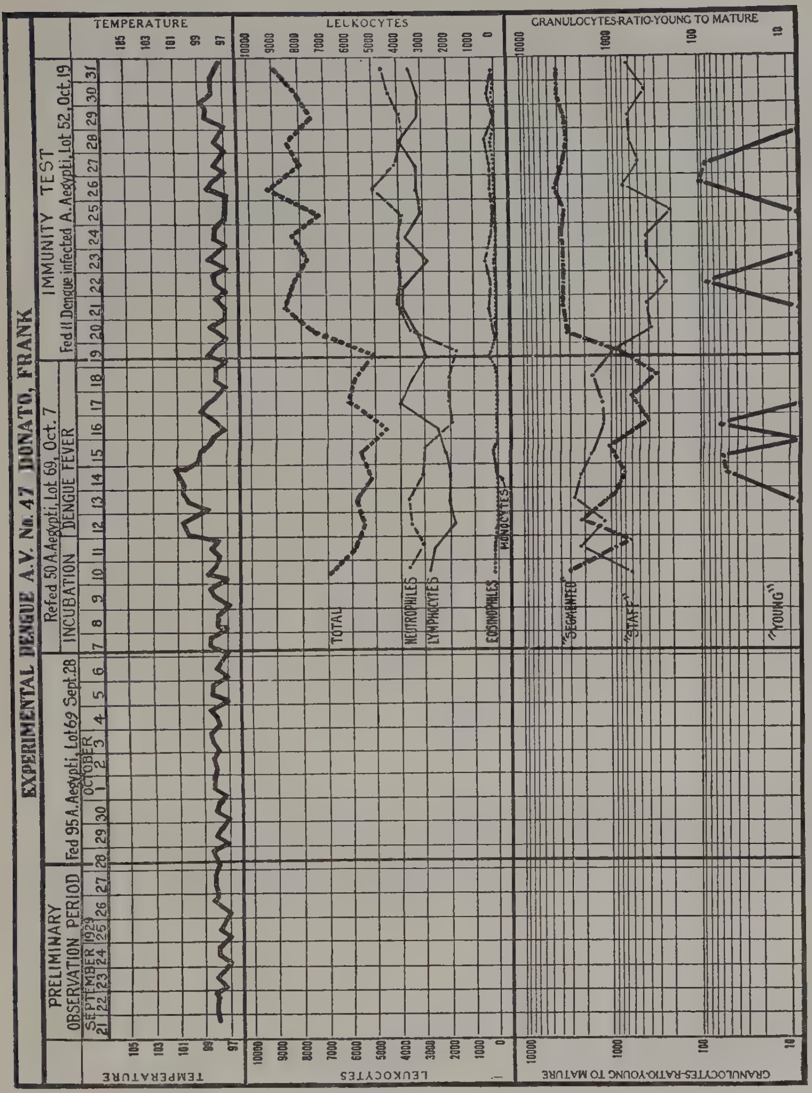

Fig. 16. Experimental dengue in American volunteer 47.

DISCUSSION

The results obtained in the experimental attempts to pass the virus of dengue directly from mosquito to mosquito seem to indicate that the degree of concentration of the virus in the mosquito-blood mixture used to infect normal *Aëdes* is an important factor. In the mixtures used in the successful experiments, the concentrations were about 0.5, 1.21, 3.33, and 4.05 mosquitoes, respectively, per 0.1 cubic centimeter of fluid, while in the unsuccessful tests the mixtures contained about 0.058, 0.25, 0.45, and 3.33 mosquitoes to 0.1 cubic centimeter of fluid. As it has been shown that a single *A. ægypti*, during a normal feeding, ingests about 0.00253 gram, or about 0.002 cubic centimeter, of blood, it may be presumed that during the successful experiments the normal mosquitoes each ingested about 0.012, 0.031, 0.083, and 0.102, respectively, of one infected mosquito, while in the unsuccessful experiments each normal mosquito ingested 0.0015, 0.006, 0.011, and 0.083 *A. ægypti*. The most-dilute infective suspension used successfully was that employed in experiment 8, in which each normal mosquito ingested material representing about 0.01 of an infected mosquito; and at least 7 per cent of the lot fed on this material were unable to transmit dengue.

The possibility of decreasing the virulence of dengue virus by serial mosquito passages is of interest. As was noted in experiments 1, 4, and 6, all of which dealt with primary transfers of virus from mosquito to mosquito, one of the volunteers developed a severe case of dengue, two had average cases, and one person had a mild infection. The one case of dengue caused by mosquitoes infected by a second serial transfer of virus was milder than usual. Thus, while the virulence of dengue virus is apparently not modified by the first passage from infected to normal *A. ægypti*, there is a possibility that serial passages through mosquitoes might result in decreased virulence of the virus.

It should be noted that in experiments 6 and 8 the mosquitoes which fed on volunteers five and seven days after the ingestion of virus failed to transmit dengue, while the same lots did infect volunteers when tested on a later date. Thus, it would appear that the virus from infected mosquitoes, as that obtained from warm-blooded animals, must pass through a period of development or multiplication in *Aëdes* before the mosquito can infect man through feeding.

SUMMARY AND CONCLUSIONS

1. The virus of dengue has been transferred from infective to normal mosquitoes by feeding normal *Aëdes ægypti* on infected mosquitoes macerated and suspended in normal blood.

2. Successful passage of dengue virus in this manner was obtained with a mixture containing not less than five infective mosquitoes per cubic centimeter of blood.

3. The virus apparently retained its virulence for man after the initial passages from mosquito to mosquito. There is a possibility that the virulence of dengue virus might be decreased after serial passages through mosquitoes.

4. A period of more than seven days was required before normal mosquitoes that had ingested dengue virus from mosquito suspensions could transmit dengue to man.

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ATTEMPTS TO DEMONSTRATE THE VIRUS OF DENGUE BY MICROSCOPIC EXAMINATION

For many years workers have searched for the organism responsible for dengue fever. McLaughlin(1) in 1886 published an account of his investigations concerning the etiology of dengue and reported having found a spherical micrococcus which was one-twentieth to one-thirtieth the diameter of an erythrocyte, both in the fresh and incubated blood of dengue cases and in nutrient gelatin cultures made with the blood. Graham(2) in 1903, working in Syria, described an organism seen within red blood cells which, except for the presence of pigment, resembled *Plasmodium malariæ.* He reported having found these bodies in the stomachs of *Culex fatigans* up to the fifth day following their feeding on dengue patients, and also the finding of spores of the organism in the salivary glands of the mosquito. Later workers, including Carpenter and Sutton (Isthmus of Panama, 1903),(3) Guiteras and Cartaya (Cuba, 1905),(4) Report of the Bureau of Government Laboratories (Manila, P. I., 1900),(5) Agramonte (Havana, Cuba, 1906),(6) Kiewiet de Jonge and de Haan (Java, 1905),(7) Stitt (Philippine Islands, 1906),(8) and Ashburn and Craig (Philippine Islands, 1907),(9) were unable to confirm the findings of McLaughlin and Graham. Furthermore, they were unable to consistently demonstrate in the blood any organism that they could logically consider of etiological significance.

Later investigators were concerned with attempts to demonstrate in dengue-infected materials a spirochæte or *Leptospira* similar to the *Leptospira* found by Noguchi(10) in cases diagnosed as yellow fever. Couvy,(11) (1921) after direct examination of the blood of dengue patients, reported having found a short slender spirochæte; while Siler, Hall, and Hitchens(12) observed a spirochæte-like form in the blood of several dengue patients, which they considered had nothing to do with the etiology of dengue as was indicated by their careful control work with blood of normal individuals. Carbo-Noboa in 1924(13) reported finding spirochætes in the serum, citrated blood, and cerebrospinal fluid of dengue patients, examined microscopically with dark-field illumination and after the use of Levaditi's stain. Knowles and Des Gupta(14) came to the conclusion that spiral structures found in the blood of dengue patients in Calcutta, which were at first thought to be *Leptospira,* were in reality artefacts. De Faria(15) found a *Leptospira* in dengue patients in Brazil; and Vervoort(16) observed "*Leptospira pyrogones*" in a denguelike fever in India. However, Chandler and Rice (17) from experiments on man and animals obtained no evidence to indicate that the causative agent of dengue was a *Leptospira,* an observation which agrees with the results of Knowles and Des Gupta,(14) Holt,(18) Siler, Hall, and Hitchens,(12) Duval and Harris,(19) and the authors of this report. When it was shown that yellow fever is probably not due to a *Leptospira,* further search for such an organism in dengue was discouraged; and the attention of investigators has been directed into other channels.

Duval and Harris,(19) using Noguchi's(10) medium, found "globoid" bodies, measuring from 0.1 to 0.3 micron, in cultures made with dengue blood. Sellards and Siler,(20) in a study of histological sections from normal and dengue-infected *Aëdes ægypti,* found masses of *Rickettsia* in the hind gut of infected mosquitoes, but none in a control group of normal mosquitoes. The *Rickettsia* were not seen in the salivary glands or in any tissue outside of the alimentary tract. Moutoussis(21) also found *Rickettsia* and inclusion bodies in *Stegomyia* infected with dengue virus. However, Schule(22) writes that during examinations of *A. ægypti* fed on dengue blood and of others fed on normal blood, he regularly found in both groups of mosquitoes organisms identical with those described by Sellards and Siler.

It is apparent that much time has been spent in attempts to demonstrate visually an organism responsible for dengue fever; and that at various times bacteria, Protozoa, *Leptospira,* and *Rickettsia* have been suggested as of possible etiological significance. However, none of these organisms has been definitely incriminated. On the other hand, considerable evidence has accumulated to indicate that the virus, in the blood of patients and in infected mosquitoes, is probably too small to be seen by any means now available.

EXPERIMENTAL

Microscopic examination of blood from dengue patients.—Blood specimens obtained daily from two cases of experimentally induced dengue (A.V. 1–H and A.V. 2–M), during the incubation period and throughout the disease, and specimens from two others (A.V. 3–A and A.V. 4–Y), collected on each day of the disease, were prepared in the following manner and examined microscopically: (*a*) Dark-field preparations; (*b*) films stained by Wright's, Giemsa's, and Mann's methods and with flagella stains. The results were negative. A number of blood preparations were treated with 95 per cent alcohol, tannic acid, saturated solution of ammonium sulphate, formalin and copper sulphate in various dilutions, and then stained with Giemsa's stain, Wright's stain, gentian violet and other dyes, but none of the preparations contained anything recognizable as the virus of dengue.

Attempts to render the virus visible by precipitation and concentration.—Microscopic examinations were also made of infective blood and serum which had been mixed with convalescent serum and centrifugalized in the hope that agglutination or precipitation of the virus might result in masses large enough to be seen. The results of these experiments were also negative.

Microscopic examination of inoculated culture media.—Both aërobic and anaërobic cultures were attempted by inoculating blood containing dengue virus from experimental dengue patients into Noguchi's medium, N. N. N. medium, Locke-egg-serum medium for amœba, Hibler's brain medium, Francis's cystine agar, beef infusion broth enriched with equal parts of hydrocele fluid or ascitic fluid, banana broth, hormone broth (heart), brain, spleen, and bone-marrow broth. No evidence of dengue virus was obtained by microscopic examination of the incubated materials in these tubes.

Microscopic examination of infective mosquitoes.—Repeated attempts to identify the virus of dengue in saline suspensions of *A. ægypti,* were unsuccessful. Smear preparations of the salivary glands of individual dengue-infected mosquitoes, examined microscopically with dark-field illumination, and after staining by Giemsa's and Wright's methods, failed to show any form that could not be demonstrated in control normal mosquitoes. The salivary glands of dengue-infected *Aëdes* were removed in groups of ten infected *A. ægypti* each, macerated in a small amount of salt solution, and immediately examined microscopically, using the "dark-field" and various staining methods, including Giemsa's and Wright's. No forms were seen in these preparations that could not also be found in materials from normal mosquitoes.

Microscopic examinations of serum contaminated by infective mosquitoes.—As the virus of dengue escapes from the proboscis of infected mosquitoes during feeding, an attempt was made to collect a concentrated suspension of virus by feeding 100 infective *A. ægypti*, lot 72, on a small amount of guinea-pig serum (1 cubic centimeter) that had been diluted with an equal amount of 0.9 per cent salt solution and placed in a shallow cell beneath a clean piece of skin which had just been removed from a young chicken. As a control on this procedure, approximately one hundred fifty normal *A. ægypti* were allowed to feed on another mixture of serum and salt solution similarly arranged. After the mosquitoes had fed, the serum was removed from beneath the skin and centrifugalized at 2,000 revolutions per minute for thirty minutes. Direct microscopic examination was made of the wet sediment, using both the ordinary and dark-field illumination. Dry films were stained with hæmatoxylin and eosin, and by Wright's, Giemsa's, Gram's and Hiss's (capsule) methods. Minute coccoid and rod-shaped forms were observed in all of the preparations, from both the control and the test serum, but these forms appeared to be much more numerous in the latter.

CONCLUSIONS

1. The virus of dengue was not identified by microscopic examinations of the following materials: (*a*) Blood from cases of experimentally induced dengue, (*b*) materials from culture media inoculated with the virus, (*c*) crushed *A. ægypti* infected with virus, (*d*) the salivary glands of infective *A. ægypti*, or (*e*) clear guinea-pig serum on which one hundred infective *A. ægypti* had fed.

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CULTIVATION EXPERIMENTS WITH THE VIRUS OF DENGUE

Many attempts have been made to cultivate the causative agent of dengue; but, so far, no one has furnished convincing proof that this virus is able to multiply outside of the living tissues of its hosts. McLaughlin(1) in 1886, using the limited methods of that period, studied cultures inoculated with the blood of dengue patients; but his preparations were contaminated with skin cocci and the results are of historical interest only. In 1907 Ashburn and Craig(2) approached the problem, "with high hopes that by applying modern cultivation methods we might be able to isolate and cultivate the organism causing the disease. In view of the success attained by Novy and others in the cultivation of trypanosomes, and by Rodgers in cultivating the Leishman-Donovan body (*Herpetomonas donovani*), we were especially hopeful that by applying similar methods in this disease, we might be able to secure growths of any protozoön which might be present." These investigators made cultures of dengue blood collected in citrate solutions at various periods of the disease and incubated them at different temperatures; cultures were also made by adding 10 cubic centimeters of blood to 250 cubic centimeters of bouillon in flasks. As a result of their experiments it was concluded that no organism was found constantly enough in the cultures to warrant regarding it as the cause of dengue. In 1914 Lavinder and Francis(3) also failed to demonstrate any growth of virus in cultures prepared by inoculating dengue blood into glucose broth, and incubating the tubes at 37° C. and at room temperature for several days. Chandler and Rice(4) in 1923, using methods which had proven successful with *Leptospira*, were unable to observe any growth of dengue virus. In 1924 Duval and Harris,(5) using Noguchi's medium, reported having grown minute filterable anaërobic globoid bodies, which after inoculation into guinea pigs gave rise to reactions similar to those that they had observed following injections of blood from dengue cases. While these workers considered that the globoid bodies might have had an etiological relationship to dengue they recognized that further proof was required before this could be established. Siler, Hall, and Hitchens(6) in 1926 attempted to grow the organism of dengue in various media, including (*a*) the rabbit kidney ascitic fluid of Smith and Noguchi, (*b*) the semisolid *Leptospira* medium of Noguchi, and (*c*) the peptone-acid-phosphoricum of Vervoort. These media, inoculated with blood specimens from forty-five dengue patients and incubated at 37° C. or at room temperature for intervals as long as one month, failed to show any recognizable gross or microscopic evidence of growth of the virus of dengue. Schule(7) during 1927 also made cultures with infective dengue blood inoculated into various types of media. He isolated a minute, filter-passing, "Gram-doubtful" coccobacillus in the cultures from one case. However, injection of this organism into susceptible human volunteers failed to cause dengue or to produce any immunity to the disease; and he, therefore, considered his cultivation experiments to be negative. Copanaris(8) and Manoussakis,(9) during the recent (1927–1928) epidemic in Athens, also failed to cultivate the virus of dengue.

In spite of the formidable array of negative experiments reported by previous workers, cultivation of dengue virus was again attempted by the authors, using different types of media and new methods for determining the presence or absence of virus in cultures.

PRESENT INVESTIGATION

The cultivation experiments may be divided into two main groups. In the first group various culture media, including Noguchi's serum medium, Boeck and Drbohlav's amœba medium, Hibler's brain medium, brain agar, N. N. N. medium, Francis's cystine agar, dextrose infusion broth, and mosquito broth, were inoculated with potentially infected materials from dengue patients, incubated aërobically and anaërobically at 37° C. and at room temperature, and observed both macroscopically and microscopically for evidence of growth. In the second group of experiments, it had been planned to test the cultures for the presence of virus, even though there was no visual evidence of growth, by passing the incubated material through diatomaceous filters followed by injection of the filtrates into susceptible volunteers. This procedure was not carried out because of variable results obtained in filtration experiments with known infected materials. However, the development of a satisfactory method(10) for feeding *Aëdes ægypti* artificially on infected materials, placed in a shallow cell beneath a piece of guinea-pig or chicken skin, made it possible to test the cultures for virus in this manner.

1. CULTURE MEDIA INOCULATED WITH INFECTIVE MATERIALS AND LATER EXAMINED MACROSCOPICALLY AND MICROSCOPICALLY FOR EVIDENCE OF THE GROWTH OF DENGUE VIRUS

Experiment 1.—January 2 to 12, 1929. Fresh specimens of urine and blood were collected early in the incubation period, and on the first, second, and fourth days of dengue fever, from an experimental case (A.V. 1–H). Normal *Aëdes ægypti* fed on this man on the second day of the fever and called lot 3 were later proved to be infected with the virus. After inoculating 0.1 cubic centimeter amounts of the material into the various media mentioned above, sets of both aërobic and partially anaërobic cultures (under paraffin oil) were incubated at 37° C. and at room temperature for several weeks. All of the tubes were observed daily, and microscopic examinations of dark-field preparations and of films stained by Wright's and Gram's methods were made at frequent intervals. A few miscellaneous bacterial contaminants grew in some of the tubes inoculated with urine, but none showed any evidence of dengue virus.

Experiment 2.—January 2 to 12, 1929. Fresh specimens of urine and blood collected early in the incubation period and on the second, third, fourth, and fifth days of dengue fever, from an experimental case (A.V. 2–M), were inoculated into culture media and observed as in experiment 1. The blood collected from this man on the second day of fever was infective as was proved later by passage of the virus through *A. ægypti* (lot 2). No evidence of growth of the virus was observed in these tubes.

Experiment 3.—February 25, 1929. Cultures were prepared similarly with blood immediately after its collection in a small amount of sodium citrate from an experimental case (A.V. 3–A) on the morning of the second day of dengue fever. The infectivity of this blood was indicated by the fact that *A. ægypti* fed on this man at the same time (lot 9) later transmitted dengue. Additional aërobic and anaërobic cultures were prepared by inoculating flasks containing 100 cubic centimeters of dextrose infusion broth with 0.2 cubic centimeter amounts of the citrated blood. After three weeks of negative observations the inoculated materials were discarded.

Experiment 4.—February 27, 1929. Similar tests were made with fresh citrated blood collected in the forty-ninth hour of dengue fever from an experimental case (A.V. 4–Y). The fact that normal *A. ægypti* designated lot 10, which fed on this man at the same time, were later proved infected indicates that the blood inoculated in the media also contained dengue virus. The cultural results were negative.

Experiment 5.—April 14, 1929. Fresh blood was collected in citrate from an experimental case (A.V. 6–R) during the first day of dengue; and 0.5 cubic centimeter amounts were inoculated into tubes of the following media: (*a*) Noguchi's medium, (*b*) N. N. N. medium, (*c*) cystine agar, and (*d*) beef-infusion broth. Similar inoculations were made (*e*) into flasks containing 100 cubic centimeters each of 1 per cent dextrose broth. It may be assumed that the blood contained virus, since normal *A. ægypti* fed on the patient, at the time of its collection, were later used to transmit dengue (lot 12). However, no evidence of development of the virus was observed in the cultures.

Experiment 6.—May 15, 1929. Fresh blood was collected in citrate, from a dengue case (A.V. 9–D) during the second day of the fever. The infectivity of the blood was proved by feeding normal *A. ægypti* (lot 19), which were later used to transmit dengue. The blood was inoculated into culture media as in experiment 5 and was incubated and observed as usual. The results were negative.

Experiment 7.—April 29, 1929. Fresh citrated blood was collected from a dengue case (A.V. 10–H) early on the second day of fever. Normal *A. ægypti* fed on the patient at the same time were later proved infected (lot 17). The dengue-infected blood was inoculated into media in the usual manner, but evidence of growth of dengue virus was not observed during a period of one month.

Experiment 8.—July 16, 1929. *Attempts to cultivate dengue virus in a mosquito-broth medium.* Because of the readiness with which normal *A. ægypti* may be infected with dengue virus, and the apparent ease with which the virus continues to develop in these mosquitoes after infection, it was considered possible that a culture medium containing mosquito protein might favor growth of the virus in vitro. Therefore, a large number of normal *A. ægypti* were killed, weighed, and macerated in a sterile mortar with sufficient Locke's solution to make a 2 per cent suspension, and the mosquito suspension was then sterilized by filtration. Because of the limited supply of material, this medium was used in 0.9 cubic centimeter amounts in long slender tubes prepared from narrow glass tubing. After inoculation with 0.1 cubic centimeter amounts of the infected serum or blood, the tubes were incubated at 37° C.; and at two- to four-day intervals 0.1 cubic centimeter amounts were transferred to fresh tubes of the medium. The blood used in this experiment was collected in sodium citrate from a case (A.V. 22–M) early on the second day of dengue; and was proved infective at the time by *A. ægypti* (lot 30) and *A. albopictus* (lot 29). The inoculated tubes of media were examined daily during a period of two weeks, but there was no evidence of growth of virus.

Experiment 9.—September 6, 1929. Fresh citrated infective blood drawn from a dengue case (A.V. 34–S) on the second day of fever was inoculated over the surface of seven thin slices each of (*a*) boiled banana and (*b*) raw banana. Examination of the cultures for a period of two weeks failed to show any evidence of the growth of dengue virus.

2. CULTURES INOCULATED WITH INFECTIVE MATERIALS AND LATER TESTED FOR GROWTH OF VIRUS BY FEEDING TO NORMAL AËDES ÆGYPTI

In the following experiments an attempt was made to cultivate the dengue virus in a number of different types of special media. Material from the inoculated tubes was transferred serially at different intervals of time so that the final tubes contained such great dilutions of the original inoculum as to decrease the possibility of obtaining positive results by preservation of small amounts of virus rather than by its multiplication. As it had been shown that normal *A. ægypti* might be infected by feeding on dengue virus suspended in normal blood and placed in a shallow feeding cell, advantage was taken of this method for determining the presence or absence of virulent dengue virus in cultures. Therefore, after inoculation of the medium with dengue-infected materials and incubation of the transferred culture materials, this was fed to normal mosquitoes, which were later tested for infectivity by feeding on susceptible volunteers. The following culture media were used: (*a*) Galloway's relapsing-fever spirochæte medium;(11) (*b*) Locke-egg-serum medium for amœbæ; (*c*) N. N. N. medium; (*d*) hydrocele fluid; (*e*) hormone (heart) broth; (*f*) spleen broth; (*g*) brain broth; (*h*) bone-marrow broth; (*i*) banana broth; and (*j*) lymph-gland broth containing bone. These media were prepared as follows:

(*a*) *Galloway's medium for relapsing-fever spirochætes.*—Small amounts of whole-egg emulsion were placed in test tubes, and heated in a water bath until coagulated; 5 cubic centimeters of rabbit serum, which had been heated for half an hour at 58° C. and then diluted 1 to 5 with physiologic salt solution, liquid petrolatum, and 1 drop of normal blood were then added to each tube.

(*b*) *Boeck and Drbohlav's Locke-egg-serum medium for amœbæ.*(12)—Sodium chloride 9 grams, calcium chloride 0.24 gram, potassium chloride 0.42 gram, sodium bicarbonate 0.20 gram, and dextrose 2.50 grams were dissolved in 1,000 cubic centimeters of distilled water, filtered, and autoclaved at 15 pounds pressure for fifteen minutes. Four whole eggs for each 50 cubic centimeters of solution were added, the medium tubed, and coagulated in slants. Each slant was then covered in whole or part by inactivated horse serum diluted with eight parts of Locke's solution. The medium was then incubated at 37° C. to test sterility.

(*c*) *"N. N. N." medium.*—Fourteen grams of agar and 6 grams of salt (NaCl) were dissolved in 900 cubic centimeters of distilled water by autoclaving at 15 pounds for one hour; 33⅓ per cent fresh defibrinated rabbit blood was added and the medium tubed in slants. The tubes were sealed with paraffined stoppers.

(*d*) In experiment 5 the medium was hydrocele fluid combined with equal parts of veal-infusion broth.

(*e*) *Hormone (heart) broth. Modification of Huntoon's method.*—Five hundred grams of finely minced beef heart, 500 cubic centimeters of distilled water, 10 grams of peptone, 5 grams of salt (NaCl), and one whole egg well beaten were mixed together, heated to 70° C., filtered through a wire strainer, titrated, and adjusted to p_H 7.6; heated in "Arnold" one hour and after standing for several hours the clear supernatant fluid was syphoned, tubed, and sterilized by the fractional method.

(*f*) *Spleen broth.*—Five hundred grams of finely ground bovine spleen were placed in 1,000 cubic centimeters of distilled water, heated in the

"Arnold" for one hour, and strained through cotton. One per cent peptone and 0.5 per cent salt (NaCl) were added, the reaction adjusted to p_H 7.6, tubed, and autoclaved at 15 pounds pressure for one hour.

(*g*) *Brain broth.*—Five hundred grams of minced calf brain were placed in 500 cubic centimeters of distilled water, sterilized in "Arnold" for one hour, 1 per cent peptone and 0.2 per cent salt (NaCl) were added and the reaction was adjusted to p_H 7.6; it was filtered through cheese cloth and cotton until the medium was clear, and sterilized at 15 pounds pressure for thirty minutes.

(*h*) *Bone-marrow broth.*—Five hundred grams of macerated fresh bovine bone-marrow were placed in 1,000 cubic centimeters of distilled water, containing 10 grams of peptone, 5 grams of salt (NaCl), and a whole egg well beaten. The mixture was then heated to 70° C., filtered through a wire sieve and adjusted to p_H 7.6, heated in the "Arnold" for one hour, allowed to stand over night, and the clear supernatant fluid was then pipetted, tubed in 10 cubic centimeter amounts, and sterilized fractionally at 15 pounds pressure.

(*i*) *Banana broth.*—Three hundred grams of fresh ripe banana were macerated, 200 cubic centimeters of distilled water added, heated, and cleared by centrifugalization. After the reaction was adjusted to p_H 7.4, the medium was tubed and sterilized fractionally.

(*j*) *Lymph-gland broth containing bone.*—Ten grams of peptone (Fairchild's) and 5 grams of sodium chloride in 100 cubic centimeters of distilled water were heated to boiling to dissolve, were cooled, and 500 grams of finely macerated normal bovine lymph glands were added. After storage at 18° C. for twenty-four hours, the medium was autoclaved, the reaction adjusted to p_H 7.6 and filtered through paper for clearing, tubed in 9.9 cubic centimeter amounts in test tubes, which contained a small piece of fresh, clean guinea-pig bone. The medium was then covered with liquid petrolatum and autoclaved.

EXPERIMENTS

Experiment 1.—September 11, 1929. Fresh citrated infective blood withdrawn from an experimental case of dengue (A.V. 38–H) whose symptoms began in the afternoon September 10, was inoculated in 0.1 cubic centimeter amounts into each of the following media: (*a*) Galloway's medium; (*b*) Locke-egg-serum medium; (*c*) equal parts of hydrocele fluid and beef-infusion broth with and without a covering of petrolatum; and (*d*) N. N. N. medium. In each medium the transplants were made as follows: Ten tubes, each containing about 5 cubic centimeters of fluid, with the exception of the N. N. N. medium, were placed in a test-tube rack. Tube 1 was inoculated with 0.1 cubic centimeter of undiluted dengue blood. After incubation for one day 0.1 cubic centimeter was transferred to tube 2, and so on daily until the tenth tube was reached. Transfers were also made in series from tube 1, at two-day intervals to tubes 3, 5, 7, and 9; at three-day intervals to tubes 4, 7, and 10; and at four- and five-day intervals to tubes 5 and 10. Thus, various incubation periods were represented by the material in the last two tubes.

Culture materials fed to mosquitoes.—September 21, 1929, or ten days later, the contents of the last two tubes (tubes 9 and 10) of each medium, were mixed with normal human citrated blood collected from A.V. 42–H and fed to mosquitoes as follows: Cultures in media "a" and "b" were fed to one hundred twenty normal *A. ægypti* designated lot 64; cultures in medium "c" were fed to twenty-four normal *A. ægypti*, which became lot 65, and cultures in medium "d" were fed to twenty-nine normal *A. ægypti* designated lot 66.

TEST FOR VIRUS IN MOSQUITOES FED ON CULTURES

Test A.—October 7, 10.30 a. m., or sixteen days after feeding on the various culture-blood mixtures, eighty-two *A. ægypti* of lot 64, twenty-four of lot 65, and nineteen of lot 66 fed on a susceptible volunteer (A.V. 48–H). Dengue did not develop during twenty days observation.

Test B.—October 15, 9.30 a. m., or twenty-four days after feeding on the mixture of cultures and blood, thirty-five *Aëdes* of lot 64, twelve of lot 65, eleven of lot 66, and two of lot 74 again fed on the same volunteer (A.V. 48–H) and again failed to cause dengue during a period of twelve days observation.

Test C.—October 19, 10 a. m., or twenty-eight days after feeding on the culture-blood mixture, twenty-six *A. ægypti* of lot 64 fed on a susceptible volunteer (A.V. 51–S). Dengue did not develop in twelve days observation, and this lot was discarded November 1.

Test D.—October 19, 10 a. m., six *A. ægypti* of lot 65 and ten of lot 66 fed on a susceptible volunteer (A.V. 52–N). Since no symptoms of dengue occurred during the following twelve days this experiment was also considered negative, and therefore this volunteer was used on the evening of October 31 for another experiment, designed to test the infectivity of a different lot of *A. ægypti* (lot 99). However, November 1 at 10.30 a. m., or twenty-four hours following the feeding of lot 99 *A. ægypti* and thirteen days after the last feeding of lots 65 and 66, A.V. 52–N developed symptoms of dengue. Eighty-eight normal *A. ægypti* were fed at this time and designated lot 105, but were not tested. Comment: The fact that this volunteer (A.V. 52–N) was absent from the ward the night of October 27, just five days before he developed dengue makes it impossible to be sure of the source of his infection. However, considering the fact that thirteen days had elapsed since the feeding of six and ten *A. ægypti*, respectively, of lots 65 and 66; and the fact that about twice as many mosquitoes of these same lots had failed to infect A.V. 48–H who was bitten by them October 7 and again October 15, it seems possible that these mosquitoes of lots 65 and 66 were not responsible for the infection.

Experiment 2.—September 21, 1929. Infected blood was collected from an experimental case of dengue (A.V. 37–M) early on the second day of fever, and was inoculated in 0.1 cubic centimeter amounts into tubes containing 9.9 cubic centimeters each of hormone broth (heart), spleen broth, brain broth, and banana broth. From these first tubes (I), which contained the original blood in dilutions of 1 to 100, immediate transfers of 0.1 cubic centimeter amounts were made to a second series of tubes (II), resulting in dilutions of 1 to 10,000; from the second 0.1 cubic centimeter was carried to a third series (III), resulting in dilutions of 1 to 1 million; and from the latter tubes 0.1 cubic centimeter amounts were transferred to a fourth series (IV), resulting in dilutions of 1 to 1 billion. After incubation at 37° C. transfers were made from tubes I, II, and III at one-, two-, and four-day intervals for eight days, as follows: From the first tube in series I, 1 cubic centimeter amounts were transferred each day until September 28, giving a dilution of 1 to 1 billion in the final tube; from the first tube in series II, 0.1 cubic centimeter amounts were transferred every second day until September 27, resulting in a final dilution of 1 to 10 billion; from the first tube in series III, 0.01 cubic centimeter was transferred on September 25, giving a final dilution of 1 to 1 billion; while tube 1 in series IV, in which the blood was already diluted 1 to 1 billion, was incubated for eight days without transfer.

Culture materials fed to mosquitoes.—September 29, after the material in the final tubes of each series had been incubated for one, two, four, and eight days, respectively, representative portions of the culture materials were pooled, mixed with equal parts of citrated normal guinea-pig blood, and fed to normal *A. ægypti.* The final cultures in hormone broth and banana broth were pooled, mixed with blood in a feeding cell, and placed in a cage containing one hundred recently emerged normal mosquitoes, only eight of which fed on the mixture. Similar unsatisfactory results were obtained with the culture material in the brain broth and spleen broth, on which only ten of one hundred mosquitoes fed. A final attempt was made to feed additional mosquitoes on the following day, but only a few ingested the culture-blood mixture. The thirty-six mosquitoes fed on these cultures were combined and designated lot 80.

Test for virus in the mosquitoes.—Twelve days later five *A. ægypti* of lot 80 fed on a newly admitted volunteer (A.V. 50–C). Dengue fever did not result in this case.

Conclusions.—The small numbers of *A. ægypti* that fed on these cultures and the smaller number left for use in testing the infectivity of the lot, rendered the negative results of this experiment inconclusive.

Experiment 3.—September 27, 1929. Three series of tubes were prepared containing, respectively, (*a*) brain broth, (*b*) spleen broth, and (*c*) bone-marrow broth. The first tube in each series was inoculated with 1 cubic centimeter of citrated infected blood drawn thirty minutes earlier from a patient (A.V. 42–H), who had developed symptoms of dengue fever twenty-four hours previously. Thereafter, transfers were made daily to fresh tubes of media resulting in the following dilutions of the original blood: Brain-broth and spleen-broth mediums.—Tube 1 (1–10), 2 (1–100), 3 (1–2,000), 4 (1–40,000), 5 (1–800,000), 6 (1–160 millon), 7 (1–320 million), 8 (1–64 billion). Bone-marrow broth.—Tube 1 (1–10), 2 (1–100), 3 (1–1,000), 4 (1–20,000), 5 (1–200,000), 6 (1–2 million), 7 (1–20 million), 8 (1–200 million).

Culture materials fed to mosquitoes.—October 5, a mixture was prepared by adding 5 cubic centimeters of defibrinated normal human blood to 1 cubic centimeter each of the bone-marrow broth (1–200 million), brain broth (1–6.4 billion), and spleen broth (1–6.4 billion) culture materials. After placing this culture-blood mixture in a feeding cell, seven normal *A. ægypti* fed and were designated lot 75. After an additional two days incubation another mixture was prepared from the cultures as follows: One cubic centimeter bone-marrow broth (1–200 million), 2 cubic centimeters bone-marrow broth (1–20 million), 1 cubic centimeter spleen broth (1–64 billion), and 1 cubic centimeter brain broth (1–64 billion) to which was added 5 cubic centimeters of defibrinated normal human blood. Forty-eight normal *A. ægypti,* which fed on this mixture, were added to the seven mosquitoes remaining in lot 75.

Test for virus in mosquitoes.—October 21, 10 a. m., fourteen *A. ægypti* of lot 75 and one of lot 80 fed on a susceptible volunteer (A.V. 50–C). Dengue did not develop.

Conclusion.—There was no indication that the virus of dengue grew in these cultures.

Experiment 4.—December 4, 1929. Blood containing virus was obtained from an experimental case (A.V. 62–H) during the beginning of dengue. Normal *A. albopictus* which fed at the same time, designated lot 128, later produced dengue in two monkeys (*M. philippinensis* 30 and 31) and in A.V. 69–K. This infective blood was immediately inoculated in 0.1 cubic centimeter amounts into tubes containing approximately 6 cubic centimeters each of the following media: (*a*) Equal parts of hydrocele fluid and veal-infusion broth (*b*) the same medium covered with paraffin oil, and (*c*) Boeck and Drborhlav's amœba medium. After incubation at 37° C., serial transplants of 0.1 cubic centimeter amounts were made December 5, 7, 9, 11, and 13. December 17, the final cultures in each medium were mixed with equal parts of guinea-pig blood, making a total of about 15 cubic centimeters of fluid, a part of which was then fed to normal *A. ægypti.*

TESTS OF THE MOSQUITOES FOR INFECTIVITY

Test A.—The normal *A. ægypti* fed on the hydrocele-fluid medium were called lot 140. Fifty mosquitoes of this lot later fed on a susceptible volunteer (A.V. 67–McC) at 9.30 a. m., January 13, 1930, but failed to cause dengue. This lot was retested at 10 a. m., January 24, 1930, when thirty-four mosquitoes again fed on a susceptible volunteer (A.V. 77–E) and again failed to produce dengue. The lot 140 mosquitoes were considered uninfected and were discarded February 12, 1930.

Test B.—Normal A. *ægypti* fed on the hydrocele-fluid medium incubated under paraffin, and designated lot 141, were tested along with lot 140. Thirty of these mosquitoes fed on A.V. 67–McC January 13, and twenty-one fed on A.V. 78–O January 24, 1930. As dengue did not develop in either of these susceptible volunteers the lot 141 mosquitoes were considered uninfected, and were discarded February 12, 1930.

Test C.—Normal *A. ægypti* fed on the cultures in the amœba medium were designated lot 142 and tested on a susceptible volunteer (A.V. 71–W). Ninety mosquitoes fed on this man January 13, 1930; and seventy again fed January 27, 1930. As the results were negative these mosquitoes were discarded February 13, 1930.

Conclusion.—There was no evidence indicating growth of the dengue virus in these cultures.

Experiment 5.—December 4, 1929, 10.45 a. m. Blood was collected from an experimental case (A.V. 62–H) early on the second day of dengue and 0.1-cubic-centimeter amounts were inoculated into tubes containing 5 cubic centimeter amounts of the following media: (*a*) Brain broth, (*b*) brain broth covered with paraffin oil, (*c*) spleen broth, (*d*) spleen broth covered with paraffin oil, (*e*) hormone broth (heart), (*f*) hormone broth covered with paraffin oil, (*g*) bone-marrow broth, (*h*) bone-marrow broth covered with paraffin oil. Each culture was incubated at 37° C., and at two-day intervals 0.1 cubic centimeter amounts were transferred serially to new tubes of media.

Culture material fed to mosquitoes.—December 23, 1929, each final culture was mixed with equal parts of normal guinea-pig blood and fed artificially to normal *A. ægypti* which were designated as follows: One hundred thirty-eight mosquitoes fed on the brain broth (*a*), lot 147; one hundred thirty mosquitoes fed on the brain broth (*b*), lot 148; one hundred three fed on spleen broth (*c*), lot 149; one hundred forty fed on spleen broth (*d*), lot 150; sixty fed on hormone broth (*e*), lot 151; one hundred fifty fed on hormone broth (*f*), lot 152; one hundred thirty-eight fed on bone-marrow broth (*g*), lot 153; and ninety-six mosquitoes fed on bone-marrow broth (*h*), lot 154.

Test for virus in mosquitoes.—These various lots of *A. ægypti* were tested for dengue virus as follows:

Test A.—January 13, 1930, ninety-three, ninety-eight, eighteen, and one hundred two mosquitoes of lots 147, 149, 151, and 153, respectively, fed on a susceptible volunteer (A.V. 71–W) and failed to cause dengue.

Test B.—January 27, 1930, eighty-five, seventy-five, twelve, and one hundred sixteen mosquitoes of the same lots again fed on A.V. 71–W with negative results.

Test C.—January 13, 1930, eighty-one, one hundred twelve, ninety-five, and eighty-seven *A. ægypti* of lots 148, 150, 152, and 154, respectively, were fed on a susceptible volunteer (A.V. 68–J) and failed to cause dengue.

Conclusion.—There was no evidence of the growth of dengue virus in any of these cultures.

Experiment 6.—December 16, 1929. Defibrinated blood was collected from a dengue patient (A.V. 66–W) during the first day of fever and inoculated in 0.1 cubic centimeter amounts into tubes containing 9.9 cubic centimeters each of lymph-gland broth. Transfers were made serially of 0.1 cubic centimeter amounts at two-day intervals for twenty-eight days. January 15, 1930, the final culture was mixed with an equal amount of normal guinea-pig blood and fed artificially to one hundred fifty-five normal *A. ægypti*, called lot 155. This lot was tested at 10.30 a. m., February 15, 1930, when twelve of the mosquitoes fed on a susceptible volunteer (A.V. 79–H). As the volunteer did not develop dengue from this biting, the mosquitoes were considered to be free from virus.

Comment.—No evidence was obtained to indicate that dengue virus was cultivated in the media used.

DISCUSSION

The results of these cultivation experiments agree with the negative observations of other workers and indicate that dengue virus, as it exists in infected blood, is probably incapable of multiplication in vitro in the culture media used. However, as the etiological agent of dengue has not yet been recognized morphologically and its presence is detected only by demonstrating its ability to cause typical infection in man, the evidence at hand is hardly sufficient to prove that dengue virus failed to reproduce in these experiments. For example, it is at least possible that the virus added to the initial tubes of media might have developed and been carried by transfer to the final tube, but that due to some change during cultivation it had become incapable of infecting the normal mosquitoes by which it was ingested. It is also conceivable that the virus might have developed in virulent form in all of the cultures, but that due to slow growth or to its great dilution in the final tubes of media, it was not present in sufficient concentration to cause infection of the mosquitoes. However, the latter possibility is considered to be unlikely. It has been shown by the authors that during an average complete feeding normal *A. ægypti* ingest about 2.5 milligrams of blood; and that this amount of blood from a dengue patient, or a similar amount of a mixture containing five infective *A. ægypti,* suspended in 1 cubic centimeter of normal blood, is sufficient to infect normal mosquitoes. Since relatively large numbers of mosquitoes took full feedings from the cultures, it appears that the chances favored their infection, had the virus been present even in small amounts. For example, one hundred *A. ægypti,* each ingesting 2.5 milligrams from 5 cubic centimeters of equal parts of normal blood and culture material spread in a thin layer with an area of 3 by 4 inches, would result in the testing of one hundred different samples of 2.5 milligrams each, representing a total of 250 milligrams of the culture-blood mixture. Therefore, in spite of the unexplained result obtained in test D, experiment 1, it seems likely that the virus of dengue failed to multiply in the media used, as is indicated by the negative results obtained in the other transmission experiments.

CONCLUSIONS

1. In these cultivation experiments no macroscopic or microscopic evidence was observed indicating growth of the virus of dengue in vitro.

2. The negative results of the tests on susceptible volunteers to determine the possible infection of *A. ægypti* that had fed on inoculated media indicate that dengue virus failed to grow in the culture media used.

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THE VIABILITY OF DENGUE VIRUS

Information concerning the viability of dengue virus under different conditions is of considerable importance; however, because of the rather difficult and tedious technical procedures necessary for the detection of dengue virus, such information is limited and is largely incidental to various other experimental observations considered to be of more immediate importance. The available information indicates that dengue virus is able to exist for relatively long periods in the bodies of certain infected mosquitoes, for shorter periods in infected human beings and lower animals, and for very short periods outside the living bodies of these hosts.

Viability of dengue virus in Aëdes mosquitoes.—It has been shown by Siler, Hall, and Hitchens,(1) Blanc and Caminopetros,(2) and the present authors that dengue virus remains alive in infected *A. ægypti* for periods of at least seventy-five, one hundred seventy-four, and seventy days, respectively; and the authors have recently shown that the virus lives for at least fifty-four days in *A. albopictus*. Apparently, the living tissues of these mosquitoes furnish ideal conditions for the continued existence of dengue virus, which probably lives until the death of the insect. While there is very little information available concerning the duration of life of the virus in infected mosquitoes after their death, it has been shown by filtration experiments with suspensions of killed *A. ægypti* that the virus remained alive, at room temperature, for at least two hours. However, attemps to demonstrate virus in the dried tissues of *A. ægypti* after longer periods were not successful, as is shown in the following experiments.

Experiment 1.—March 3, 1930. One hundred infective *A. ægypti*, including sixty-nine of lot 162, nine of lot 135, seven of lot 137, five of lot 138, and ten of lot 139, were killed, ground in a mortar, desiccated over calcium chloride, and stored in a vacuum in a sealed glass tube, kept in the refrigerator.

After four days storage (March 7) the mass of dry mosquito tissue was suspended in 3 cubic centimeters of a mixture consisting of 1 cubic centimeter of physiologic salt solution and 2 cubic centimeters of defibrinated normal human blood. One hundred twenty-four normal *A. ægypti* fed on this mosquito suspension and were designated lot 179. March 22 and 24, 1930, sixteen of these lot 179 mosquitoes were tested for infectivity by feeding on a susceptible volunteer (A.V. 91–K). Dengue did not result.

Experiment 2.—March 3, 1930, one hundred infective *A. ægypti* of lot 164 were killed, dried, and stored in a manner similar to that followed in experiment 1. Four days later the material was mixed with blood and fed to sixty-seven normal *A. ægypti* which were designated lot 180. Twelve of these mosquitoes fed on a susceptible volunteer (A.V. 91–K) March 24, 1930, but failed to cause dengue.

Comment.—While these two negative experiments fail to prove that the virus had died after being kept for four days in the dried tissues of dead *A. ægypti*, it seems probable that this was the case.

Viability of dengue virus in blood.—After virulent virus has been introduced into susceptible human beings it remains alive throughout the incubation period of from four to ten or more days, and during at least the first three, and probably more, days of the infection. This is no doubt true also of infections in susceptible lower animals, particularly monkeys.

Thus, the life of the virus in man may be estimated at from seven to fifteen days, with the possibility that very minute amounts of virulent virus or larger amounts of attenuated virus may exist for much longer periods. It is apparent that the small amount of virus introduced by a mosquito during the infective biting multiplies more or less rapidly, until within four to ten days it is present in sufficient amount to cause symptoms of dengue fever. During the day preceding the fever the concentration of virus in the blood may or may not be great enough to cause infection in normal mosquitoes which ingest full feedings of about 0.025 gram of the blood.

Mosquitoes fed on the first and second days of the disease practically always become infected, indicating that at this time the concentration of infective virus is at its height. On the third day of fever there is probably a diminution in virulent virus, as mosquitoes may or may not be infected by feeding at this time; while on subsequent days mosquitoes can rarely be infected by feeding on the blood of dengue patients. However, from the reports of various investigators who have produced dengue by inoculating volunteers with large amounts of blood collected from patients at different periods, it appears possible that while diminished in amount virulent virus may be present throughout the fever. As there is no method available for the recognition of attenuated virus, it is, of course, also possible that during infection the virus may not be killed but may continue to live and multiply in an attenuated form, which might be transmissible to insects and might even revert to the infective state at a later period. While this possibility is purely speculative there is no available experimental evidence to indicate that the virus of dengue is killed in man or that a deferred carrier state might not occur in this host.

VIABILITY OF VIRUS IN INFECTIVE BLOOD AFTER ITS COLLECTION FROM DENGUE PATIENTS

Cleland, Bradley, and McDonald(3) (1919) produced dengue in volunteers by inoculations of infective blood which had been kept at refrigerator or room temperatures for periods of 48, 172, and 99 hours, respectively. Koizumi, Yamaguchi, and Tonomura(4) (1918) reported that in two instances dengue virus in infective blood, which was diluted with three and four volumes of saline, was infective after standing for five hours at room temperature, while similar materials kept in the ice chest gave negative results when injected into volunteers. Blanc and Caminopetros(5) (1929) found that the addition of one-fifteenth volume of bile to dengue blood rendered the virus non-infective within five minutes, while smaller amounts caused a decrease in virulence. Manoussakis(6) (1928) preserved virus for six days by collecting 25 cubic centimeters of infective blood during the first twenty-four hours of dengue, and mixing this with 200 cubic centimeters of normal saline, which was then sealed and stored in an incubator. Six days later the subcutaneous inoculation of 6 cubic centimeters of the supernatant fluid caused dengue. He also observed that the infective power of dengue virus in serum was destroyed by heating to 50° C. for half an hour, while exposure to 40° C. for a similar period did not interfere with its virulence. Virus was not destroyed by exposure to sunlight for half an hour, or by storage for seven days at 37° C., or at room temperature for eight days. Blanc, Caminopetros, and Manoussakis(7) (1928) announced that dengue virus in serum, stored in sterile flasks at room temperature, remained infective for a period up to two months. Although the observations of various workers concerning the viability of dengue virus after removal from the living tissues of its hosts differ considerably, it is apparent that under favorable conditions the virus may live for several days. Although somewhat resistant to unfavorable conditions, the many attempts made to cultivate dengue virus have been unsuccessful; and as yet no one has been able to prove that it can multiply in vitro.

The recent announcement by Sawyer, Lloyd, and Kitchen(8) (1929), of a method by which yellow-fever virus may be preserved for at least one hundred fifty-four days by freezing the infective blood of monkeys immediately after collection, drying it in a vacuum while frozen, and storing the dried blood in sterile glass containers kept in the refrigerator, led to the following experiments in which an effort was made to preserve dengue virus in a similar manner.

ATTEMPTS TO PRESERVE DENGUE VIRUS IN FROZEN DRIED BLOOD

Blood was collected from experimental dengue-fever patients during the first day of the disease, and 0.5 to 1 cubic centimeter amounts were immediately placed in sterile Petri plates, evaporating dishes, or small test tubes, and frozen at once, either in an ice-brine mixture or in solid carbon dioxide. The frozen blood was then placed in a desiccator surrounded by ice and brine, and was dried over calcium chloride until the following day when it was sealed in pyrex glass tubes. After storage at 18° C. for different intervals of time, portions of the samples of dried blood were suspended in physiologic salt solution and tested for the presence of infective virus by subcutaneous inoculation into susceptible volunteers. As indicated in Table 1, no evidence was obtained to show that any of these preparations contained infective dengue virus after storage for periods of four, fifteen, fifty-two, and eighty days at ice-box temperatures.

SUMMARY AND CONCLUSIONS

1. From the available information it is evident that infective dengue virus remains alive in its mosquito and animal hosts for much longer periods than in the dead tissues of these hosts, but that the duration of life in the latter under favorable conditions may be not inconsiderable.

2. The virus of dengue was apparently not preserved in an infective state in desiccated infective mosquitoes stored at 18° C. for four days or in frozen dried blood collected from experimental cases of dengue during the first day of fever when stored at 18° C. for four, fifty-two, and eighty days.

TABLE 7.—*Attempts to preserve dengue virus in frozen dried blood.*

Source of infective blood.			Period of storage in ice box.
Date.	Dengue patient.	Day of disease.	
			Days.
July 17, 1929	A.V. 21–M	First	4
July 17, 1929	do	do	4
June 26, 1929	A.V. 14–A	do	15
June 26, 1929	do	do	52
Sept. 10, 1929	A.V. 38–H	do	80

Subsequent test of dried blood for dengue virus.						
Date of inoculation into volunteers.	Volume of original inoculated.	Suspended in—	Filtered or unfiltered.	Susceptible volunteer.	Days observations after inoculation.	Result.
	cc.				*Days.*	
July 21, 1929	0.5	Salt solution	Filtered	A.V. 24–H	21	Negative.
July 21, 1929	0.5	do	Unfiltered	A.V. 26–T	13	Do.
July 11, 1929	1.5	do	Filtered	A.V. 28–P	24	Do.
Aug. 17, 1929	1.0	do	Unfiltered	A. V. 32–W	14	Do.
Nov. 29, 1929	1.0	do	do	A.V. 65–F	18	Do.

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FILTERABILITY OF DENGUE VIRUS OBTAINED FROM INFECTED MOSQUITOES

It was first demonstrated by Ashburn and Craig(1) in 1906 that infective virus from the blood of individuals suffering with dengue may be passed through diatomaceous filters. This observation has since been confirmed by other workers. For example, Koizumi, Yamaguchi, and Tonomura(2) in 1917 were successful in one of four tests; and Cleland, Bradley, and McDonald(3) in 1919 were able to pass dengue virus through filters in three of six experiments. Thus, it is apparent that while the virus, as it exists in the blood of dengue patients, is filterable, it is also to be noted that owing to several variable technical factors, filtration experiments with dengue virus, as with other viruses, are not always successful.

The present study was made to determine the filterability of the virus obtained from infected mosquitoes with the idea in mind that the results might give some indication as to whether the virus is similar during its development in both the insect and vertebrate hosts, or whether a different developmental cycle is required in the mosquitoes; also, if the virus obtained from the mosquitoes proved to be consistently filterable, it was planned to take advantage of this method for removing bacteria from suspensions of insects of various species fed on dengue blood, in order to test them for the presence of virus by inoculation into volunteers.

EXPERIMENTAL

METHODS AND MATERIALS

(*a*) *Source of dengue virus.*—The dengue virus fed to the various mosquito lots used in these experiments was a virulent strain (K) obtained originally in December, 1928, from a patient, Keller, who had dengue. This strain had been continued for a long period of time by repeated passages through man and mosquitoes.

(*b*) *Infection of mosquitoes.*—The mosquitoes of each lot were first given full feedings of infective blood from experimental cases of dengue during the first or second day of the disease.

In every instance the blood was proved infective at the time of feeding, by later transmission of the disease to susceptible volunteers either by mosquitoes of the same lots or, in the case of *Culex quinquefasciatus,* by *Aëdes* which had ingested the blood at the same time. *Aëdes ægypti* was used in all but two experiments, while *A. albopictus* was selected for one and *C. quinquefasciatus* for another.

(*c*) *Preparation of mosquito suspensions.*—In preparing the material for filtration, the mosquitoes were killed, and after having been ground up in a sterile mortar with sterile sand or powdered pyrex glass, they were suspended either in physiologic salt solution or in hormone broth.

(*d*) *Filtration.*—The suspensions of mosquitoes were immediately passed through cotton or were centrifugalized, to remove skeletal structures; and were then filtered through either Mandler or Berkefeld candles at a negative pressure of from 300 to 600 millimeters. The filters, which were examined for defects and tested to determine the pressure necessary to produce bubbles immediately after immersion in water, were found to differ considerably in resistance. Filtration of the mosquito suspensions required from ten to forty-five minutes. In every instance cultures showed the filtrates to be free of *Bacillus pyocyaneus* and the other culturable bacteria normally present in the mosquitoes.

(*e*) *Test for dengue virus in the filtrates.*—The various mosquito filtrates were tested for the presence of dengue virus by subcutaneous inoculation into susceptible volunteers, within two hours of the beginning of the experiments.

Experiment 1.—April 20, 1929. Fifty *Aëdes ægypti* of lot 10 were selected for this test. Mosquitoes of this lot had ingested infective blood fifty-two days previously from an experimental case (A.V. 4–Y) during the first day of dengue, and had transmitted dengue to volunteer A.V. 5–M. The mosquitoes were killed, placed in a sterile porcelain mortar, and macerated with 8 cubic centimeters of physiologic salt solution. This suspension was immediately passed through a "preliminary" type of Mandler filter (our No. 1) at a negative pressure of about 600 millimeters. When tested immediately afterwards it prevented the passage of a young culture of *Micrococcus melitensis.* The entire 6 cubic centimeters of filtrate obtained was then injected subcutaneously into a susceptible volunteer (A.V. 10–H), who after an incubation period of eight days, developed typical dengue fever, as is indicated in fig. 17. The diagnosis in this case was confirmed by subsequent transmission of the virus through *A. ægypti* of lot 17 to another volunteer (A.V. 12–S). Comment: In this experiment dengue virus from a saline suspension of *A. ægypti,* lot 10, passed through a "preliminary" type of Mandler filter.

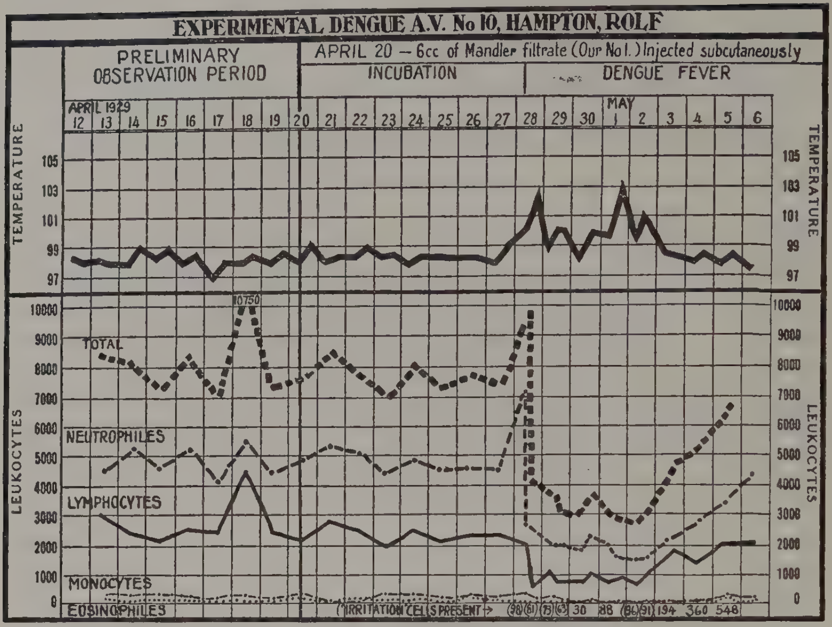

Fig. 17. Experimental dengue in American volunteer 10.

The positive results obtained in the first experiment suggested the possibility that suspensions of dengue virus from infected mosquitoes might be consistently filtered. Consequently, experiments were planned in an attempt to determine (*a*) whether or not the virus in *A. ægypti* is filterable and infective prior to the time when the mosquito is capable of transmitting the disease, and (*b*) whether or not the virus of dengue might not be developed and be demonstrable in the tissues of *Culex quinquefasciatus* even though mosquitoes of this species should be unable to transmit the infection.

Filtration experiments 2, 3, 4, and 5 were conducted with groups of mosquitoes from *A. ægypti* lot 25, all of which had taken full feedings of infective blood from an experimental case (A.V. 14–A) during the first twelve hours of dengue. At intervals of three, five, and fifteen days after the ingestion of this blood, groups of the mosquitoes were killed and suspended in salt solution. The filtrates obtained were inoculated subcutaneously into susceptible volunteers, while control mosquitoes from the same groups were allowed to feed on other volunteers. The infectivity of lot 25 was proved by the fact that after allowing the usual period for development of the virus, these mosquitoes fed on two men (A.V. 21–M and A.V. 22–M) and produced dengue in both instances.

Experiment 2.—June 29, 1929. Forty dengue-infected *A. ægypti* of lot 25, which had *fed on infected dengue blood only three days previously*, were ground in a mortar with sterile sand and suspended in 8 cubic centimeters of physiologic salt solution. After filtration through a wisp of cotton to remove the gross particles, this material was passed at a negative pressure of about 400 millimeters through a "preliminary" type of Mandler filter (our No. 10) which allowed air to bubble at about 4.4 pounds. Because of an accidental exposure of the material to possible bacterial contamination, it was filtered a second time. Five cubic centimeters of filtrate was obtained and 3 cubic centimeters of this was immediately injected subcutaneously into a susceptible volunteer (A.V. 20–B). Dengue did not result from this inoculation. Comment: As was to be expected, the control "biting" experiment with thirteen other "3-day" mosquitoes of this lot was negative, although dengue virus was known to have been present. Therefore, it seemed probable that failure of this filtration experiment might have been due to the fact that three days after feeding on infected blood the virus in the mosquitoes was not in a filterable stage, or that it was present in such small amounts as to be absorbed and retained in the filter, or that the technical procedure used and the double filtration might have prevented the passage of dengue virus regardless of its amount and source.

Experiment 3.—July 1, 1929. Forty dengue-infected *A. ægypti* of lot 25, which *had ingested dengue blood five days previously*, were ground up in a mortar with sterile sand and suspended in 6 cubic centimeters of physiologic salt solution. Filtration was carried out as in experiment 2, at a negative pressure of about 600 millimeters, through a "preliminary" type of Mandler filter (our No. 10), but the material was filtered only once. Three cubic centimeters of the filtrate was injected subcutaneously into a volunteer (A.V. 23–B), but failed to produce dengue. Feeding experiments with sixty-one additional "5-day" mosquitoes of the same lot also gave negative results. Comment: In this experiment the saline suspension was passed through the filter only once, rendering it improbable that double filtration was the only factor responsible for the negative result obtained in experiment 2.

Experiment 4.—July 11, 1929. One hundred fifty infective *A. ægypti* of lot 25 were used. These mosquitoes, which *had ingested infective blood fifteen days previously*, were proved to be infective, as they transmitted dengue by feeding to two susceptible volunteers (A.V. 21–M and A.V. 22–M). The one hundred fifty mosquitoes were ground in a mortar with sterile sand and suspended in 10 cubic centimeters of physiologic salt solution. This was divided into three portions of 4, 4, and 2 cubic centimeters, respectively, and one of the 4 cubic centimeter portions was used in this experiment. After adding 2 cubic centimeters of physiologic salt solution, the suspension, which represented sixty infected mosquitoes, was passed at a negative pressure of about 600 millimeters through a "preliminary" type of Mandler filter (our No. 10) which allowed air to bubble through at 4.4 pounds positive pressure. One cubic centimeter amounts of this filtrate were injected subcutaneously into each of two susceptible volunteers (A.V. 20–B and A.V. 23–B), but in both instances dengue failed to develop. Comment: In this experiment a saline suspension of virulent dengue virus from infective *A. ægypti* failed to pass through a "preliminary" type of Mandler filter. Therefore, it appeared probable that the negative results in experiments 2 and 3 were due to differences in technic or in the porosity of the filter candles, rather than to any variation in the filterability of the virus at different stages in the mosquito.

Experiment 5.—July 11, 1929. The 2 cubic centimeter portion of the suspension of *A. ægypti*, lot 25, mentioned under experiment 4, was diluted with 1 cubic centimeter of salt solution and passed at a negative pressure of about 600 millimeters through a coarse Berkefeld filter (our No. 3), which when tested later allowed air to bubble through at 6.8 pounds positive pressure. One cubic centimeter amounts of the bacteria-free filtrate were immediately injected subcutaneously into each of two susceptible volunteers (A.V. 24–H and A.V. 25–D), but in both instances they failed to produce dengue. Comment: In this experiment a saline suspension of virulent dengue virus from infective *A. ægypti* failed to pass through a coarse Berkefeld filter.

Experiment 6.—July 20, 1929. Twenty-four *A. ægypti* of lot 25, which had *ingested dengue blood twenty-four days previously*, and had been proved infective by feeding experiments, were combined with six other *A. ægypti* from infected lots 19 and 22 and suspended in 5 cubic centimeters of physiologic salt solution. Half of this mosquito suspension was passed at a negative pressure of 600 millimeters, through a "preliminary" type of Mandler filter (our No. 7), which when soaked in distilled water and tested immediately after use allowed air to bubble through at about 3.8 pounds positive pressure. Two and three-tenths cubic centimeters of the bacteria-free filtrate were injected subcutaneously into a susceptible volunteer (A.V. 20–B); and failed to cause dengue. Comment: The virus in a saline suspension of mosquitoes failed to pass through a Mandler filter (our No. 7).

Experiment 7.—July 20, 1929. The remaining 2.5 cubic centimeter portion of saline suspension of dengue-infected *A. ægypti*, mentioned under experiment 6, was passed at a negative pressure of 600 millimeters through a "preliminary" type of Mandler filter (our No. 8). This filter, when tested after immersion in distilled water July 13, allowed air to bubble through at a positive pressure of 6.4 pounds. The bacteria-free filtrate was injected subcutaneously into a susceptible volunteer (A.V. 23–B) and did not cause dengue. Comment: The infective virus in this saline suspension of mosquitoes failed to pass through a Mandler filter (our No. 8).

Experiment 8.—July 29, 1929. Thirteen dengue-infected *A. ægypti* of lot 25, which *had ingested dengue blood thirty-three days previously*, were combined with nineteen infected *A. ægypti* of lot 30 and eighteen of lot 32 and were suspended in about 10 cubic centimeters of salt solution. The material from the suspension of these fifty mosquitoes was passed at 400 millimeters of vacuum through a preliminary Mandler filter (our No. 7). About 6 cubic centimeters of bacteria-free filtrate was obtained and 4 cubic centimeters of this was injected subcutaneously into a susceptible volunteer (A.V. 27–P), but dengue did not result. Comment: In this experiment infective virus failed to pass through a preliminary Mandler filter.

Two other lots of mosquitoes, *A. albopictus* lot 29, and *A. ægypti* lot 30, were used later in filtration experiments 9 and 10. These mosquitoes had ingested blood from an experimental case (A.V. 22–M) July 16, during the latter half of the first day of the disease. Both of these lots of mosquitoes transmitted dengue later when numbers of them were fed on susceptible volunteers.

Experiment 9.—July 21, 1929. Fifteen *A. albopictus* of lot 29, which had *fed on infective dengue blood five days previously,* were macerated with sterile sand and 1.5 cubic centimeters of salt solution. The suspension was filtered at a negative pressure of about 600 millimeters through a regular Mandler filter (our No. 9), which when tested July 12 had allowed air to bubble through at a positive pressure of 6.4 pounds. Half of the bacteria-free filtrate (0.75 cubic centimeter) was immediately injected subcutaneously into a susceptible volunteer (A.V. 27–P) with negative results. Comment: This Mandler filtrate did not contain virulent dengue virus.

Experiment 10.—July 21, 1929. Seven *A. ægypti* of lot 30, which had *fed on infective dengue blood five days previously,* were macerated with sand and 3 cubic centimeters of salt solution. This suspension was filtered at a negative pressure of about 600 millimeters through a "preliminary" grade of Mandler filter (our No. 7). One and one-half cubic centimeters of the bacteria-free filtrate were injected subcutaneously into a susceptible volunteer (A.V. 25–D), with negative results. Comment: This Mandler filtrate did not contain infective dengue virus.

In experiments 11 and 12 the mosquitoes used were *Culex quinquefasciatus* of lot 31, and *Aëdes ægypti* of lot 32. Both had ingested dengue-infected blood from the same experimental case (A.V. 21–M) during the first day of the disease. Lot 32 *A. ægypti* were later proved to be infected, as they were able to transmit the disease to a susceptible volunteer by feeding.

Experiment 11.—July 20, 1929. Twenty-five *C. quinquefasciatus* of lot 31, which had *ingested infective blood four days previously,* were killed, macerated with sterile sand, and suspended in 4 cubic centimeters of physiologic salt solution. The mosquito suspension was filtered at about 600 millimeters negative pressure through a "regular" type of Mandler filter (our No. 12), which when tested on the same day allowed air to bubble through at 6.6 pounds positive pressure. Three cubic centimeters of the bacteria-free filtrate was injected subcutaneously into a susceptible volunteer (A.V. 28–P) with negative results. Comment: The fact that this filtrate was free from infective dengue virus does not prove that the *C. quinquefasciatus* of lot 31 did not harbor the virus, as filtrates from *A. ægypti* known to be infected were also free from virus in several instances.

Experiment 12.—August 17, 1929. One hundred infective *A. ægypti* of lot 32, which *thirty-one days previously had fed on dengue blood* similar to that ingested by *C. quinquefasciatus* lot 31, were killed, freed of wings and legs, and ground in a sterile glazed porcelain evaporating dish with 4 cubic centimeters of hormone broth. The suspension was centrifugalized at low speed for five minutes to remove gross particles and was divided into two portions of 2 cubic centimeters each. One of these portions was passed at about 300 millimeters negative pressure, through a "preliminary" type of Mandler filter (our No. 7), after the filter had been saturated by drawing hormone broth through it. About 5 cubic centimeters of filtrate was obtained, and half of this (2.5 cubic centimeters) was inoculated subcutaneously into a susceptible volunteer (A.V. 30–L), but the injection failed to produce dengue. Comment: The use of hormone broth in this experiment did not make it possible to pass the virus through the filter.

Experiment 13.—August 17, 1929. The other 2 cubic centimeter portion of the mosquito suspension in hormone broth, described under experiment 12, was passed, at about 300 millimeters negative pressure, through a Berkefeld "V" filter (our No. 19), which after immersion in water allowed air to bubble at about 9.3 pounds positive pressure. The 2 cubic centimeters of mosquito suspension was added, a drop at a time, to the top of the candle, and as none of it came through the filter in fifteen minutes, an additional 3 cubic centimeters of hormone broth was added in an attempt to wash the virus through. As a result 2 cubic centimeters of bacteria-free filtrate was obtained, of which 1.5 cubic centimeters was immediately inoculated subcutaneously into a susceptible volunteer (A.V. 29–J). After an incubation period of nine and one-fourth days this volunteer developed symptoms of typical dengue fever. The specific nature of the infection was further proved by subsequent passage of the virus through *A. ægypti* lot 49 to another volunteer (A.V. 33–B). Conclusions: In this experiment dengue virus from the hormone-broth suspension of *A. ægypti* lot 32 passed through a "V" (coarse) type of Berkefeld filter.

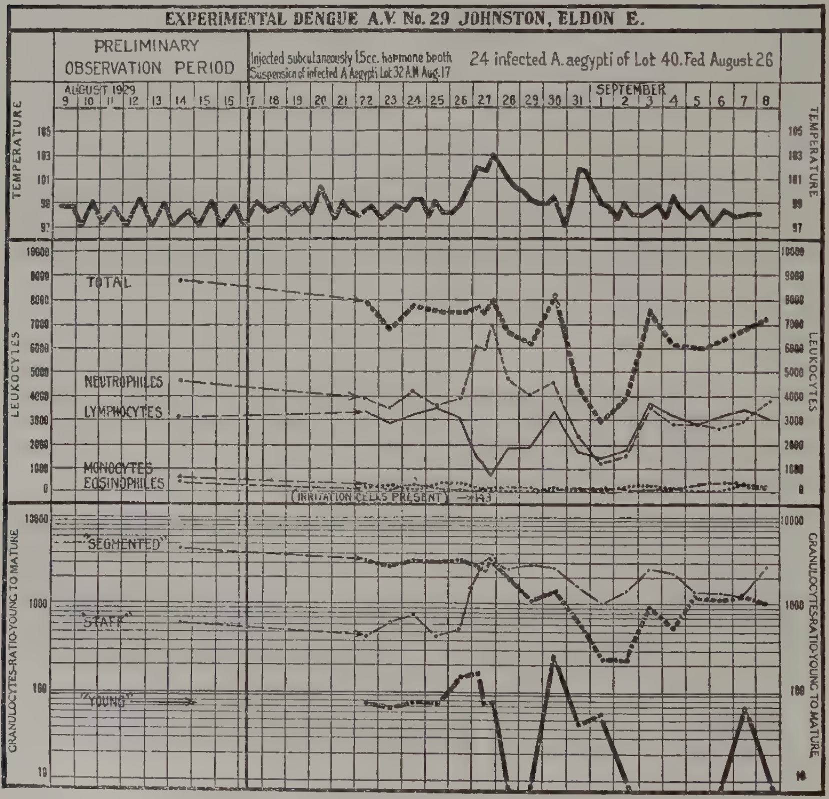

FIG. 18. Experimental dengue in American volunteer 29.

Experiment 14.—November 29, 1929. Fifty dengue-infected *A. ægypti* of lot 95, which thirty-three days previously had ingested dengue blood from an experimental case (A.V. 48–H), were killed, ground up, and suspended in 4 cubic centimeters of hormone broth. The material was passed at 600 millimeters negative pressure through a "regular" type of Mandler filter (our No. 23) in ten minutes. One cubic centimeter of the bacteria-free filtrate was injected subcutaneously into a susceptible volunteer (A.V. 64–C). Dengue did not result from this inoculation.

Experiment 15.—November 30, 1929. Fifty infected *A. ægypti* of lot 104, which *twenty-nine days previously* had fed on blood from an experimental case (A.V. 55–S) during the first day of dengue and had later transmitted dengue by biting, were killed, ground up with powdered pyrex glass, and suspended in 2 cubic centimeters of hormone broth. The suspension was centrifugalized slowly for one minute and the supernatant fluid was passed at 600 millimeters negative pressure through a Berkefeld W filter (our No. 20) which allowed air to bubble at 12.4 pounds pressure. After about thirty minutes an additional 1 cubic centimeter of hormone broth was passed through the filter. The entire time of filtration was forty-five minutes and the total amount of filtrate 1.5 cubic centimeters. Injection of 1 cubic centimeter of this bacteria-free filtrate failed to produce dengue in a volunteer (A.V. 63–B), but as this volunteer was later found to be immune the experiment has no significance.

Experiment 16.—March 14, 1930. Thirty-one dengue-infected *A. ægypti* of lot 168 and sixty-nine of lot 167 were killed, ground up with powdered pyrex glass, and suspended in 5 cubic centimeters of physiologic salt solution. The suspension was placed in the mantle of a Berkefeld V filter (our No. 19) which allowed air to bubble through at 9.3 pounds pressure, and suction (600 millimeters) was applied for five minutes. Then 3 cubic centimeters of salt solution was added and about 1.5 cubic centimeters of filtrate was obtained in five minutes. One cubic centimeter of the bacteria-free filtrate was injected subcutaneously into a susceptible volunteer (A.V. 87–R). After nine and one-quarter days incubation, dengue developed from this inoculation, as shown in fig. 19. Comment: Dengue virus from this saline suspension of *A. ægypti* passed through a Berkefeld V filter.

DISCUSSION

It has been shown by these experiments that the virus of dengue as it exists in infective mosquitoes may, under certain circumstances, be passed through diatomaceous earth filters. However, as has been observed in filtration experiments with the blood of dengue patients and with materials containing other "filterable" viruses, such positive results were not obtained in all tests.

Because of the many variable factors which might have influenced the results, it was not possible to determine with exactness just what combination of conditions are necessary to ensure

TABLE 8.—*Filterability of dengue virus from mosquitoes (Berkefeld and Mandler filters).*

Experiment No.	Mosquitoes used.		Preparation of suspension.			Filtration of suspension.			Filtrate.[a]		
	Species.	Days since ingestion of virus.	Number of mosquitoes used.	Amount of physiologic salt solution.	Amount of harmone broth.	Filter used.	Negative pressure used.	Time required.	Amount obtained.	Amount injected into volunteer.	Results of injection.
				cc.	*cc.*		*mm.*	*Min.*	*cc.*	*cc.*	
1	*Aëdes ægypti*	52	50	8		Mandler No. 1 (preliminary)	600	30	6	6	Dengue fever in 8 days.
2	do	3	40	8		Mandler No. 10 (preliminary)	400	20	5	3	Negative.
3	do	5	40	6		do	600	20	4	3	Do.
4	do	15	60	6		do	400	25	4	1 1	Do. Do.
5	do	15	30	3		Berkefeld No. 3 "V"	600	25	2.5	1	Do.
6	do	24 67 63	12 1 2	2.5						1	Do.
						Mandler No. 7 (preliminary)	600	15	2.3	2.3	Do.
7	do	24 67 63	12 1 2	2.5		Mandler No. 8 (regular)	600	20	1.8	1.5	Do.
8	do	33 13 12	13 19 18	10		Mandler No. 7 (preliminary)	400	20	6	4	Do.
9	*Aëdes albopictus*	5	15	1.5		Mandler No. 8 (regular)	600	20	1	0.75	Do.
10	*Aëdes ægypti*	5	7	3		Mandler No. 7 (preliminary)	600	15	1.8	1.5	Do.
11	*Culex quinquefasciatus*	4	25	4		Mandler No. 12 (regular)	600	20	3.3	3	Do.
12	*Aëdes ægypti*	31	50		2	Mandler No. 7 (preliminary)	300	15	[b] 5	2.5	Do.
13	do	31	50		[c] 2	Berkefeld No. 19 "V"	300	25	2	1.5	Dengue fever in 9½ days.
14	do	33	50		4	Mandler No. 23 (regular)	600	10	2	1	Negative.
15	do	29	50		[d] 2	Berkefeld No. 20 "W"	600	45	1.5	1	Negative (immune).
16	do	29 16	69 31	8		Berkefeld No. 19 "V"	600	10	1.5	1	Dengue fever in 9½ days.

[a] No demonstrable growth was obtained in culture of the filtrates.

[b] In experiment 12 the filter was first saturated with hormone broth, and drained as completely as possible before use; it is apparent that the filter still contained broth in addition to that used in the mosquito suspension.

[c] Three added later.

[d] One added later.

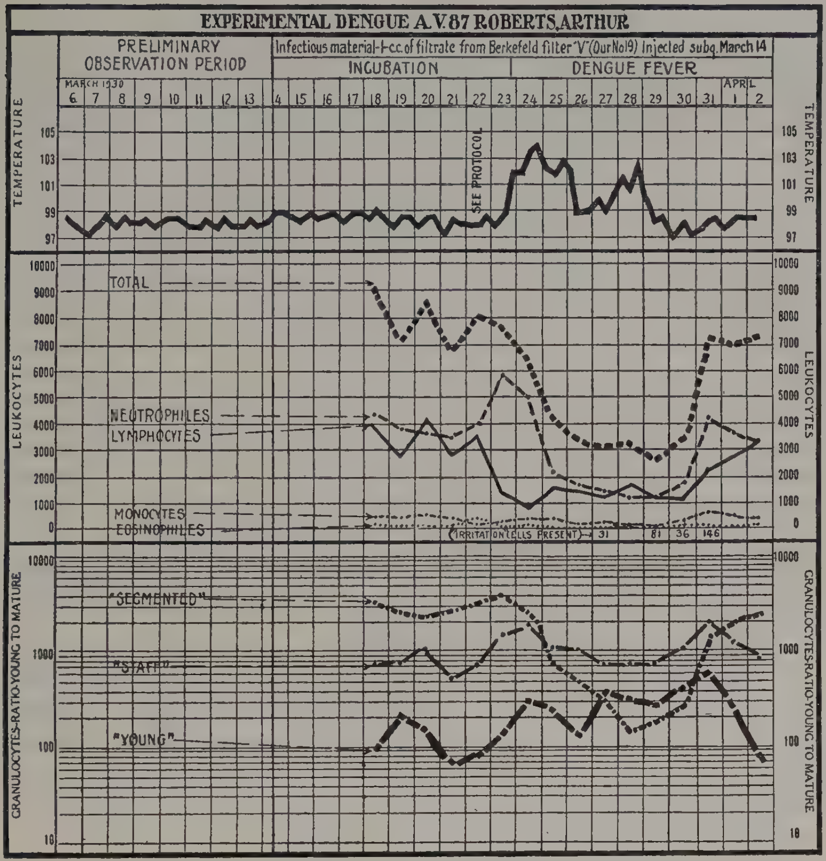

FIG. 19. Experimental dengue in American volunteer 87.

filtration of the virus at all times. It will be noted in Table 8 that in the three successful filtration experiments the conditions were as follows: (*a*) In the first test fifty mosquitoes which had been infected for fifty-two days were suspended in 8 cubic centimeters of salt solution, passed at 600 millimeters through a Mandler filter, and 6 cubic centimeters of this filtrate was inoculated into the volunteer; (*b*) in the second, fifty mosquitoes were suspended in 2 cubic centimeters of hormone broth, passed at 300 millimeters through a Berkefeld "V" filter and washed through by adding 3 cubic centimeters more broth, and then 1.5 cubic centimeters of the 2 cubic centimeters of filtrate was injected into the volunteer; while (*c*) in the third test, one hundred mosquitoes suspended in 5 cubic centimeters of salt solution were passed through a Berkefeld "V" filter with an additional 3 cubic centimeters of salt solution and 1 cubic centimeter of the filtrate was used for the inoculation of the volunteer. Since the type of these filters, the negative pressure applied, the reactions, and the materials used in preparing the mosquito suspensions were duplicated in negative experiments, it appears possible that the most important factor was the relative amounts of virus present in the suspensions. It is believed that in the negative experiments the suspensions contained such small amounts of virus that they were adsorbed and held in the candles, and that the use of larger amounts of virus might have given positive results. In the three successful tests the concentration of virus was probably greater because of the larger amounts of mosquito suspensions used. In this connection it appears of some significance that in the three volunteers infected by these filtrates, the incubation periods were eight, nine and one-fourth, and nine and one-fourth days, respectively, or longer than the average. It seems reasonable to suspect that this prolongation of the period of incubation might have resulted because small amounts of virus were present in the filtrates injected.

Note.—It is of interest to add that since the first of these successful experiments was reported(4) an article has been published by Sawyer and Frobisher(5) announcing the fact that the virus of yellow fever in infected *Aëdes* mosquitoes is filterable.

SUMMARY AND CONCLUSIONS

1. Sixteen filtration experiments are reported, in which attempts were made to pass dengue virus from suspensions of infected mosquitoes through various types of diatomaceous earth filters.

2. The variable results obtained in these experiments agree in general with the observation of others concerning the filterability of dengue virus in blood.

3. In three experiments, one with a Mandler filter and two with Berkefeld "V" filters, infective virus was shown to be present in the filtrates.

4. The inoculation of these three filtrates into volunteers resulted in dengue fever after incubation periods of eight, nine and one-fourth, and nine and one-fourth days, respectively.

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EXPERIMENTAL DENGUE IN MAN

It is commonly believed that many of the fevers of the Tropics masquerade under the diagnostic term "dengue." This state of confusion is attributable mainly to the lack of any specific diagnostic test with which to differentiate dengue from similar or related fevers of undetermined origin. For this reason it is at least possible that studies based only on the clinical signs and symptoms observed in naturally acquired infections might fail to indicate the true clinical picture of dengue.

The following analysis of clinical symptoms observed in over eighty cases of experimental dengue is considered of interest and value mainly because of the fact that they were all produced by a single strain of dengue virus. While the possibility, of course, exists that more than one type of denguelike virus may exist in nature, the fact that 93 per cent of thirty-three Filipinos residing in endemic areas were shown to be immune to experimental infection, indicates that the virus used in this work is probably immunologically similar to or identical with the dengue virus prevalent in the Philippines.

The diagnosis of experimental infections produced by this virus was confirmed by the appearance of distinctive symptoms, typical leukocyte reactions, and in the majority of instances by subsequent transmission of the infection through mosquitoes to other individuals. It is of interest to note that even with this single strain of virus, wide variations occurred in the character and severity of the disease.

It was considered possible that these variations might have resulted either from differences in the amounts of virus introduced into the volunteers; from changes in the virulence of the virus due to the various experimental procedures or passages; or from individual differences in the susceptibility of the volunteers infected. It was apparent that the latter factor alone was of great importance. As infections produced in May, 1930, did not differ noticeably from others caused by the same strain of virus a year and a half earlier, it is obvious that the virulence was neither increased nor decreased appreciably. The fact that infections similar in type and severity were caused by the feeding of either small or large groups of infected mosquitoes (Table 9) indicates that within these limits, the dose of virus failed to influence the character of the symptoms. However, it was noted in certain filtration experiments, and in others where virus was transferred by the interrupted feeding of mosquitoes that the incubation periods were slightly prolonged. It was considered probable that this was due to the introduction of minute amounts of virus into the volunteers.

Therefore, it is believed that the clinical variations observed in the entire group of experimental cases were due mainly to differences in individual susceptibility of the volunteers to dengue.

THE PERIOD OF INCUBATION

It has been estimated that the period required for the incubation of dengue virus in man is from two to ten days, but Armstrong(2) states that it is usually from three to six days. Cleland(3) (1923) is of the opinion that "the incubation period of the natural disease may be set down as being usually from 3 to 8 days, with outside limits of 2½ and 15 days. Such an incubation period is consistent with clinical experience. Instances in which the time appears to have been less than 2 days can probably all be explained in some other way." He reported an incubation period of fifteen days in one volunteer who was inoculated with blood drawn from a case in the fourth day of the disease. Siler, Hall, and Hitchens,(1) (1926) from an analysis of their experimentally infected cases, arrived at the following conclusions:

> The average incubation period in our series was six and one-twentieth days; in 25 per cent it was less than five days; in 60 per cent, less than six days; in 75 per cent, less than seven days; and in 90 per cent, less than eight days. Incubation periods of more than ten days must be very rare in naturally acquired cases.

The results obtained in the present series, shown in Table 10, give an average incubation period of 5.66 days, with 83.75 per cent of eighty infections occurring between the fourth and seventh days after inoculation. The maximum period observed was between eleven and thirteen days; and the minimum was between three and four days. However, it is to be noted that only one case occurred in each of the latter groups, and that the infection with the prolonged incubation period was the result of interrupted feeding of *Culex quinquefasciatus*.

TABLE 9.—*Experimental dengue in man. A comparison of cases produced by small and large numbers of mosquitoes.*

Number of mosquitoes fed.	Lot No.	Volunteer No.	Incubation period.	Fever.		Leukopenia.		Rash.			Character of attack.		
				Duration.	Height.	Duration.	Lowest point.	None.	Primary.	Secondary.	Mild.	Average.	Severe.
			Days.	*Days.*		*Days.*							
7	9	I.V. 1–T	5.25	5.5	103.6	5	4,700	*					*
6		I.V. 2–B	5.25	4	101.2	2	4,700	*				*	
6		I.V. 3–P	5.25	6	102.4	6	4,600	*					*
5		I.V. 4–B	4?	4 ?	101	4	4,100	*				*	
9	30	A.V. 20–B	6	3.5	100.4	3.5	4,200		*		*		
8	32	A.V. 28–P	6	4.5	102.6	5	3,700	*				*	
10	52	A.V. 46–L	5.5	5.5	102.4	9	3,250			*		*	
9		A. V. 43–D	4.5	5.5	103.6	8	2,600		*?			*	
6		A.V. 41–F	4.5	6	103.2	5	2,850		*	*		*	
8		A.V. 44–F	5	5.5	101.6	4	3,300			*	*		
8	71	A.V. 55–S	5	5	104	10	2,950	*				*	
10	86	A.V. 50–C	5.5	4	104	5	2,800		*	*		*	
10	112	A.V. 65–F	6	5.5	102.4	7	3,150		*	*			*
7	137	A.V. 70–S	5	5	102.8	4	3,150		*	*		*	
6		A.V. 71–W	6	2	102.6	7	2,900			*	*		
7.7 (average)			5.25	4.8	102.4	5.7	3,530	[a] 40	[a] 40	[a] 47	[a] 20	[a] 60	[a] 20
56	2	A.V. 4–Y	7.5	3	104	2.5	3,700	*				*	
56	3	A.V. 3–A	6	5	103	5.5	3,350	*					*
85	18	A.V. 13–W	4.5	5	102	5	2,400	*				*	
139	10	A.V. 5–M	5	4.5	104	4	2,950			*			*
82	35	A.V. 31–D	4	5.5	104	9	2,500			*			*
64	71	A.V. 48–H	5	5.5	103.2	9	2,050			*		*	
109	114	A.V. 66–W	5	4	101.4	3	4,150		*	*		*	
84	116	A.V. 61–J	5	5	104	8	2,950			*			*

58	117	A.V. 64-C	6	4.5	102.2	6	2,600	----	*	----	----	*	----
89	137	A.V. 67-M	5	6	103	5	2,900	----	*	*	----	*	----
61	137	A.V. 76-M	6	3.5	101.2	7	3,300	----	----	*	*	----	----
60	138	A.V. 75-P	5	5.5	103.8	7	2,150	----	*	----	----	*	----
110	139	A.V. 68-J	5.5	5.5	103.8	5	3,400	----	*	*	----	*	----
87	162	A.V. 77-E	4.5	6.5	102	7	1,750	----	*	*	----	*	----
108	167	A.V. 82-F	4.5	6.5	101.4	3	4,100	----	----	*	----	*	----
83.2 (average)	----	----	5.2	5.0	102.9	5.7	2,937	[a] 20	[a] 40	[a] 66	[a] 6.6	[a] 66	[a] 26.6

[a] Per cent.

TABLE 10.—*The incubation periods in experimental dengue fever observed in eighty cases.*

Days of incubation.	Cases.	Per cent.
3–4	1	1.25
4–5	15	18.75
5–6	36	45.00
6–7	16	20.00
7–8	5	6.25
8–9	2	2.50
9–10	4	5.00
11–13	1	1.25
Total	80	100.00

[a] Average, 5.66 days incubation.

PRODROMAL SYMPTOMS AND ONSET

In 58 per cent of the sixty cases included in fig. 20, the onset of the disease was abrupt, beginning with fever or severe headache, or more rarely, with a distinct chill. In a few of the remaining 42 per cent of cases with gradual onset, mild prodromal symptoms such as malaise, weakness, chilliness, and indefinite pains and aches were noticed for a day or so before the definite attack. The symptoms at onset consisted of fever, headache, flushing of the face, aching of the eyes, pains in the back and extremities, and malaise. Chilly sensations or even chills were not uncommon. In certain cases an initial rash was observed. Leukopenia was not present until the first or second day following the onset, although an increase in the immature with a corresponding decrease in the mature granulocytes was not uncommonly observed on the day preceding fever.

THE FEVER

The results obtained experimentally by Siler, Hall, and Hitchens,(1) and during the present studies, show that the temperature reactions observed in dengue may differ so greatly in type, height, and duration that this symptom considered by itself has no specific, diagnostic significance. Among the experimental infections considered in this report patients were seen who had "two-day fever," "three-day fever," and so on, including a few cases with "seven-day fever." As shown in Table 11, the duration of the fever in eighty-one experimental cases was

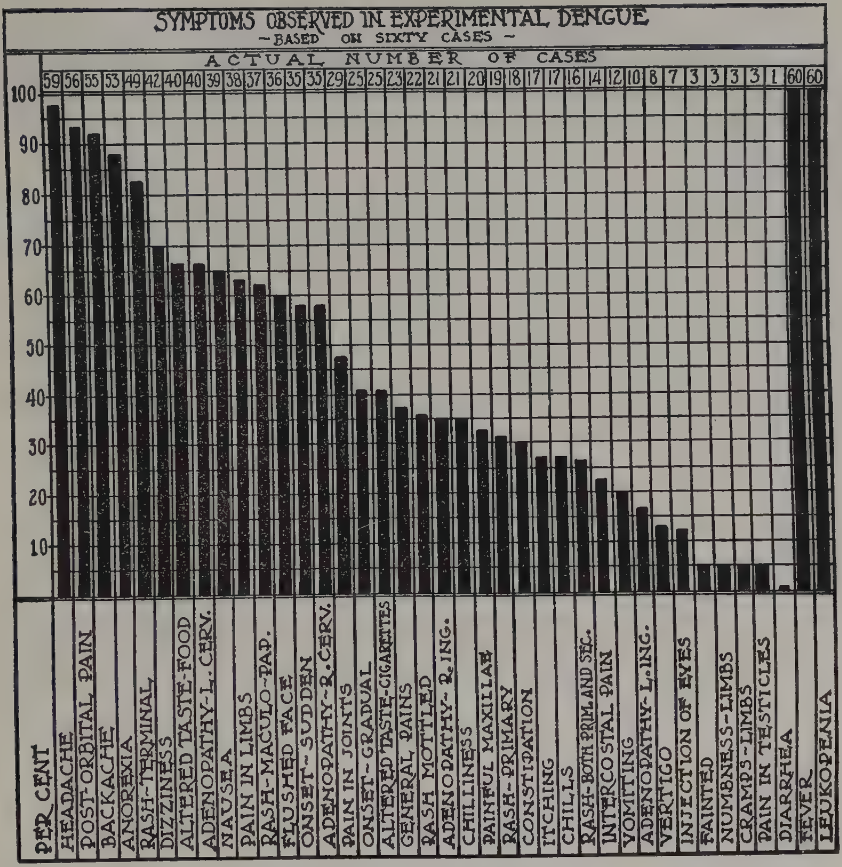

FIG. 20. **Symptoms observed in experimental dengue.**

from one to nine days, although in the average case the interval between the beginning and end of the fever was 4.8 days. There was also considerable variation in the maximum temperatures recorded. In 52 per cent the temperature was 103° F. or above, while the highest temperature reached in 48 per cent was 102.8° F. The so-called "saddle back" or intermittent type of fever, which has been considered as characteristic of dengue, was observed in certain cases but was by no means constant. In these cases the interval of reduced fever coincided with a period of relief from the subjective symptoms, while the return of the fever was usually accompanied by the symptoms noted in the first stage of the disease.

There was no indication that the fever varied in duration in any fixed relation to the incubation period, as has been observed in certain other diseases. As shown in Table 13, cases with both long and short incubation periods had fevers of similar duration.

The subjective symptoms of dengue lasted from one to nine days, during which the various symptoms noted at onset often became more pronounced. Fever, leukopenia, headache, post-orbital pain, generalized aches and pains particularly of the back, anorexia, nausea, dizziness, abnormal sense of taste, adenopathy, and terminal rash were the commonest symptoms observed. Certain of these symptoms are of sufficient importance to be considered separately.

TABLE 11.—*Duration of fever in eighty-one cases of experimental dengue fever.*

Days of fever.[a]	Cases.	Per cent.
1–2	2	2.47
2–3	3	3.71
3–4	10	12.35
4–5	16	19.75
5–6	37	45.68
6–7	11	13.58
7–8	1	1.23
8–9	1	1.23
Total	81	100.00

[a] Average, 4.8 days fever.

TABLE 12.—*Maximum temperature observed in eighty-one cases of experimental dengue fever.*

Maximum temperature.[a]	Cases.	Per cent.
°*F.*		
100–101	2	2.47
101–102	12	14.82
102–103	25	30.86
103–104	28	34.57
104–104.6	14	17.28
Total	81	100.00

[a] Average temperature 102.82° F.

TABLE 13.—*Comparison of the incubation periods and the duration of fever in eighty cases of experimental dengue.*

Incubation period.	Days of fever.							
	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9
Days.								
3–4				1				
4–5	1			1	7	5		1
5–6			4	8	20	4		
6–7		1	2	4	7	1	1	
7–8		1	2	2				
8–9					2			
9–10	1	1			1	1		
10–11								
11–13			1					
Total	2	3	9	16	37	11	1	1

PROGRESS OF THE DISEASE

Headache and generalized pains.—Beside the fever and leukopenia, which were noted in all cases, the commonest symptoms were headache, postorbital pains, and backache, which occurred in over 90 per cent of the sixty individuals considered. More than 60 per cent complained of pains in the limbs and 45 per cent had painful joints. However, while these symptoms were far from mild, none of them were severe enough to warrant application of the old name, "break-bone fever."

Skin rashes.—There seems to be a general belief among clinicians that the rash is one of the most characteristic symptoms of dengue; and consequently the clinical diagnosis frequently depends on the presence or absence of this sign. Siler, Hall, and Hitchens(1) noted that in only two, or 4 per cent, of their experimental cases was there failure to observe an eruption at some time during the course of the disease. Six of their cases had an initial eruption, but no terminal rash, and in seven instances the conditions were reversed. Twenty-three showed both initial and terminal rashes. It was stated that "evidently the eruption is a very variable symptom, requiring careful and frequent observation if all of its details are to be noted; in some cases it may fail altogether to develop."

In the present study, the initial rash, usually an erythematous flush or mottling, was observed in only 38.8 per cent of the cases (Table 14), and it was frequently difficult to differentiate this rash from other skin eruptions, particularly the heat rashes. Some of the primary rashes lasted for only a few hours, and for this reason it is considered possible that a few were not observed. Because of the difficulty experienced in recognizing the initial rash, and because of its evanescent character, this sign was not considered of definite diagnostic value. The terminal rash, which was noted in 69.5 per cent of the cases, was of more importance. It was usually of the maculo-papular variety, and in a few cases it was followed by desquamation and in some instances by itching of the palms of the hands and soles of the feet. Although this rash might be confused with other skin eruptions, its occurrence in a febrile condition, accompanied by characteristic symptoms of dengue, including leukopenia, is of considerable diagnostic significance.

Glandular enlargement.—As indicated in fig. 20, a large proportion of the cases had enlargement of the cervical or inguinal lymph glands. The cervical glands were most frequently affected, which indicates that the location of the infective mosquito bite was probably not a determining factor, since most of the volunteers were bitten on the leg. In over 30 per cent of the cases there was some tenderness in the region of the parotid glands and the patients complained that their "jaws ached." However, no enlargement of the gland was noted and the pains may have originated in the joint. Two cases were seen in Ifugao volunteers of definite parotid involvement, one of whom had such marked enlargement of the glands as to suggest temporarily that he might have mumps. Five per cent of the cases complained of swelling or tenderness of the testicles.

Gastrointestinal symptoms.—A large proportion of the patients complained of loss of appetite, and at least 65 per cent had perversions of taste for various foods or for cigarette smoke. Nausea was also common, particularly early in the disease, and vomiting occurred in 20 per cent of the cases. Constipation was noted in 30 per cent of the infections.

Genitourinary system.—In a few cases a trace of albumin was noted temporarily during the fever.

Circulatory system.—The pulse rate during the disease was usually low in proportion to the fever.

Hæmorrhage.—Among the Americans only one case of dengue had epistaxis; and none had hæmorrhage from other parts of the body. On two occasions severe nose bleed was observed in an Ifugao volunteer during the height of dengue fever.

TABLE 14.—*Experimental dengue in man.*[a]

Infection of volunteers.							Character of infection.						
Volunteer No.	Infected mosquitoes used.					Incubation period in volunteers.	Fever.		Leukopenia.		Rash.		Character of attack.
	Species.	Lot No.	Age of virus.	Number fed or injected.	Date fed or injected.		Duration.	Maximum temperature.	Duration.	Minimum count.	Primary.	Secondary.	
			Days.			*Days.*	*Days.*		*Days.*				
1	*Aëdes ægypti*	1	16	17	Dec. 31, 1928	9	2	102.4	3	3,150	×	×	Mild.
2	do.	1	15	15	Dec. 31, 1928	7	3.25	102.6	4.5	3,250	×	×	Average.
3	do.	3	40	56	Feb. 19, 1929	6	5	103	5.5	3,350	0	0	Severe.
4	do.	2	50	56	Feb. 19, 1929	7.5	3	104	2.5	3,700	0	0	Average.
5	do.	10	40	139	Apr. 8, 1929	5	4.5	104	4	2,950	0	×	Severe.
6	do.	9	45	49	Apr. 11, 1929	5	4.5	101	6	2,900	×	0	Average.
7	do.												
8	*Aëdes ægypti*	9+10	59+57	29	Apr. 25, 1929	5	5.25	103.2	6.5	2,350	0	0	Do.
9	do.	12	22	15	May 8, 1929	6	4.5	102.8	7	2,850	0	×	Do.
10	do.	10	52	50	Apr. 20, 1929	8	5.5	103.2	6	2,800	× (b)	0	Severe.
11													
12	*Aëdes ægypti*	17	12	17	May 11, 1929	6	5	102	6	2,950	0	0	Average.
13	do.	18	16	85	May 17, 1929	4.5	5	102	5	2,400	0	0	Do.
14	do.	19	37	12	May 20, 1929	5.5	5	103.8	8	2,850	0	×	Severe.
15													
16													
17													
18													
19													

[a] Average incubation period, 5.66 days; average duration of leukopenia, 6.2 days; average duration of fever, 4.8 days; primary rash, 38.8 per cent; secondary rash, 69.5 per cent; mild attacks, 13.6 per cent; average attacks, 70.4 per cent; severe attacks, 16.0 per cent.

[b] Slight.

TABLE 14.—*Experimental dengue in man*—Continued.

Infection of volunteers.							Character of infection.							
Volunteer No.	Infected mosquitoes used.					Incubation period in volunteers.	Fever.		Leukopenia.		Rash.		Character of attack.
	Species.	Lot No.	Age of virus.	Number fed or injected.	Date fed or injected.		Duration.	Maxfmum temperature.	Duration.	Minimum count.	Primary.	Secondary.	
			Days.			*Days.*	*Days.*		*Days.*				
20	*Aëdes ægypti*	30	13	9	July 29, 1929	6	3.5	100.4	3.5	4,700	×	×	Mild.
21	do	25	15	15	July 11, 1929	5	5.5	104	6	2,750	0	0	Averge.
22	do	25	15	14	July 11, 1929	4.5	5	103	7	2,150	0	0	Do.
23	*Aëdes albopictus*	29	13	12	July 29, 1929	7	4.5	101.4	6	2,150	×	×	Do.
24	do	29	22	10	Aug. 7, 1929	5.5	3.5	102	4	5,100	0	×	Mild.
25	do	29	21	3	Aug. 6, 1929	6.25	5.5	103	7	3,200	0	×	Average.
26	do	29	13	8	July 29, 1929	4.5	1.5	101	4.5	3,900	×	0	Mild.
27	do	29	21	1	Aug. 6, 1929	5	6	103.2	6	2,100	0	×	Average.
28	*Aëdes ægypti*	32	12	8	July 29, 1929	6	4.5	102.6	5	3,700	0	0	Do.
29	do	32	30	c 50	Aug. 17, 1929	9	5.5	103	7	2,950	0	0	Do.
30	do	41	20	16	Aug. 26, 1929	4.25	4	103	7	3,450	0	×	Do.
31	do	35	22	82	Aug. 26, 1929	4⅛	5.5	104	9	2,500	0	×	Severe.
32	*Aëdes albopictus*	43	14	22	Aug. 26, 1929	5	5	104.6	7	2,150	0	0	Do.
33	*Aëdes ægypti*	49	19	33	Sept. 14, 1929	4.5	6	102	6	2,500	0	0	Average.
34	*Aëdes albopictus*	43	19	2	Aug. 31, 1929	5	5	103.6	10	2,400	×	×	Severe.
35	*Aëdes ægypti*	92–*a, b, c, d, e, f, g*.	17–21	?	Aug. 26, 1929	6.5	5	102	8	3,600	×	×	Average.
36	do	44, 45, 46, 47	29–31	28	Sept. 21, 1929	5	5	103.4	7	2,750	×	×	Severe.
37	do	53	15	42	Sept. 14, 1929	7	4	103.4	7	2,700	0	×	Average.
38	do	92–*a, b, c, d, e, f, g*.	31–37	17a, 24b, 37c, 36d, 29e, 23f, 37g.	Sept. 5, 1929	5	5.5	104	9	3,100	×	×	Do.

39	do	44, 45, 46, 47	14–16	47	Sept. 5, 1929	6	6	103.4	8	2,900	×	0	Severe.
40													
41	*Aëdes ægypti*	52	37	6	Oct. 7, 1929	4.25	6	103.2	5	2,850	×	×	Average.
42	do	92–c	41–44	28	Sept. 21, 1929	5.5	6	104	11	2,650	0	×	Severe.
43	do	52	32	9	Oct. 2, 1929	4.25	5.5	103.6	8	2,600	0	×	Average.
44	do	52	41	8	Oct. 11, 1929	5	5.5	101.6	4	3,300	0	×	Mild.
45	*Aëdes albopictus*	58	26	3	Oct. 2, 1929	5	4	102	8	3,600	0	×	Average.
46	*Aëdes ægypti*	52	32	10	Oct. 2, 1929	5.5	5.5	102.4	9	3,250	0	0	Do.
47	do	69	14	50	Oct. 7, 1929	5	3.5	101	9	4,550	0	×	Mild.
48	do	71	25	64	Oct. 21, 1929	5	5.5	103.2	9	2,050	0	×	Average.
49													
50	do	86	20	10	Nov. 7, 1929	5.5	4	104	5	2,800	×	×	Do.
51	do	86	14	14	Oct. 27, 1929	5.5	6	104.4	9	2,050	0	×	Do.
52													
53	*Aëdes ægypti*	82, 83, 84, 85, 88, 89.	13–18	6, 15, 4, 6, 15, 9.	Oct. 28, 1929	6⅛	5	103.6	8	3,200	0	0	Do.
54	*Culex quinquefaciatus*	97	0	54	Oct. 31, 1929	13	3.5	103.4	6	3,100	0	×	Do.
55	*Aëdes ægypti*	71	31	8	Oct. 26, 1929	5	5	104	10	2,950	0	0	Do.
56	do	104	15	25	Nov. 16, 1929	7	2.5	100.2	5	4,350	×	×	Mild.
57	do	96	12	30	Nov. 11, 1929	5	5.5	101.6	8	2,300	×	×	Severe.
58	do												
59	do	173	29	22	Mar. 31, 1930	3⅜	4.5	101.3	4	3,950	0	0	Average.
60	do	111	(?)	103	Nov. 28, 1929	6	7.5	102.4	3	5,150	×	×	Do.
61	do	116	14	84	Dec. 7, 1929	5	5	104	8	2,950	×	×	Severe.
62	do	110	23	75	Nov. 28, 1929	4.5	6.5	103.2	6	2,600	×	0	Average.
63													
64	*Aëdes ægypti*	117	17	58	Dec. 10, 1929	6	4.5	102.2	6	2,600	×	0	Do.
65	do	112	27	10	Dec. 10, 1929	6	5.5	102.4	7	3,150	×	×	Severe.
66	do	114	24	109	Dec. 10, 1929	5	4	101.4	3	4,150	×	×	Average.
67	do	137	32	89	Jan. 27, 1930	5	6	103	5	2,900	×	×	Do.
68	do	139	41	110	Jan. 27, 1930	5.5	5.5	103.8	5	3,400	×	×	Do.

[c] Hormone broth, 7 cubic centimeters; 1.5 cubic centimeters given, Berk. "V."

TABLE 14.—*Experimental dengue in man*—Continued.

Infection of volunteers.						Incubation period in volunteers.	Character of infection.						Character of attack.
Volunteer No.	Infected mosquitoes used.						Fever.		Leukopenia.		Rash.		
	Species.	Lot No.	Age of virus.	Number fed or injected.	Date fed or injected.		Duration.	Maximum temperature.	Duration.	Minimum count.	Primary.	Secondary.	
			Days.			*Days.*	*Days.*		*Days.*				
69	*Aëdes albopictus*	128	56	14	Jan. 27, 1930	5	5.5	102.6	8	3,150	0	×	Average.
70	*Aëdes ægypti*	137	52	7	Feb. 6, 1930	5	5	102.8	4	3,150	×	0	Do.
71	do	137	52	6	Feb. 6, 1930	6	2	102.6	7	2,900	0	×	Mild.
72	do	125		11									
		126	(?)	56	Jan. 27, 1930	4	5	104	4	3,100	0	×	Average.
		145		65									
73													
74	*Aëdes ægypti*	135	17	46	Jan. 27, 1930	5.5	3.5	102.4	6	3,100	0	×	Do.
75	do	138	47	60	Feb. 3, 1930	5	5.5	103.8	7	2,150	×	×	Do.
76	do	137	46	61	Feb. 1, 1930	6	3.5	101.2	7	3,300	0	×	Mild.
77	do	162	15	87	Feb. 17, 1930	4.5	6.5	102	7	1,750	0	×	Average.
78	do	138	57	26	Feb. 12, 1930	4.5	5	103.8	7	3,050	0	×	Do.
79	*Aëdes albopictus*	160	22	118	Feb. 24, 1930	5	5	103.6	0	0	×	×	Do.
80	*Aëdes ægypti*	156	21–25	73	Feb. 15, 1930	6	4.5	102	4	3,450	0	×	Do.
81	do	157	22–26	87	Feb. 15, 1930	5.5	4.5	102.4	6	1,950	0	×	Do.
82	do	167	19	108	Mar. 4, 1930	4.5	6.5	101.4	3	4,100	0	×	Do.
83	do	168	19	11	Mar. 8, 1930	5	5	102.6	5	2,600	×	0	Do.
84	do	137	70	21	Feb. 24, 1930	4.5	8	102.4	6	3,200	0	×	Do.
85	do	164	52	5	Mar. 26, 1930	6.25	5.5	101.8	4	2,800	0	0	Do.
86	do	165	46	23	Mar. 26, 1930	5.25	5	103.6	7	2,400	0	×	Do.

87	do	159	49	3	Mar. 22, 1930	9.25	6	104.2	6	2,700	0	0	Do.
88	do	178	17	28	Mar. 22, 1930	5	3.5	103.2	2	3,200	×	×	Mild.
		181	15	19									
89	do	171	37	15	Mar. 31, 1930	5.25	4.25	103.6	7	1,900	0	×	Average.
90	*Aëdes albopictus*	183	18	15	Apr. 1, 1930	4.5	5.25	102.4	6	2,900	×	×	Do.
91	*Aëdes ægypti*	174	29	21	Apr. 1, 1930	5.25	4.5	103.8	6	4,400	0	×	Do.
92	do	189	(?)	73	Apr. 10, 1930	(?)	3.5	102.4	3	3,600	0	×	Do.
93	*Aëdes albopictus*	183	19	9	Apr. 1, 1930	9.25	1.5	101.4	6	3,600	0	×	Mild.
94	*Aëdes ægypti*	189	0	125–150	Mar. 24, 1930	7–9	5	104.6	6	3,100	0	×	Average.

The blood.—While routine erythrocyte counts were not made, no remarkable change was observed in the size, shape, color, or staining reactions of the red cells in specimens prepared daily for differential leukocyte counts. In two cases daily counts were made of the blood platelets throughout the periods of observation, incubation, and fever, but no significant variation from normal was observed.

The leukopenia and other leukocytic reactions associated with dengue are considered to be the most significant diagnostic signs of the disease. Leukopenia was observed at some time during each of the experimental infections, and after its initial appearance it was progressive, often lasting for several days after the subsidence of the fever. Because of the great importance of the leukocytic reactions to the clinical diagnosis of dengue, this subject is discussed in detail elsewhere in this report; however, it may be stated here that leukopenia, accompanied by the so-called "shift to the left" of the granulocytes, with evidence of degeneration of the mature granulocytes was observed in all of the uncomplicated cases of dengue considered in this study.

SUMMARY

The analysis of the clinical aspects of dengue fever, based on experimental cases, the majority of which were transmitted by mosquitoes, may be summarized as follows:

The average incubation period was 5.66 days; average duration of fever, 4.8 days; with primary rash, 38.8 per cent; with secondary rash, 69.5 per cent. Additional symptoms commonly observed were leukopenia, 100 per cent; postorbital pains and backache, 90 per cent; altered sense of taste, 65 per cent; pains in limbs, 60 per cent; adenopathy, 47 per cent; and pains in joints, 43 per cent.

The character of the infection in eighty-one cases may be classified as mild, 13.6 per cent; average, 70.4 per cent; and severe, 16 per cent. The onset was abrupt, usually with chill or chilliness, in 58 per cent of sixty cases considered.

It is believed that passage of the virus in series through a number of human beings and mosquitoes did not alter the virulence of the organism; that the number of mosquitoes used to transmit dengue to man, within the limits of one to one hundred thirty-nine, did not influence the character of the infection; that variations in the character of the attacks noted were probably due to individual differences in the susceptibility of the volunteers infected.

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NUMERICAL AND MORPHOLOGICAL ALTERATIONS OF THE LEUKOCYTES DURING DENGUE FEVER

It has long been recognized that typical dengue fever in man is accompanied by a characteristic decrease in the leukocytes of the blood. Attention was called to the diagnostic significance of changes in the leukocytes during dengue by Carpenter and Sutton,(1) who, in 1905, observed that a leukopenia, with a normal differential leukocytic count or varying degrees of small mononuclear lymphocytosis and a strongly marked eosinophilia late in the disease is characteristic of dengue, and that convalescence is almost invariably ushered in by a pronounced mononuclear lymphocytosis which persists for several days. These observers found the average total leukocytic counts to be about 3,500, with individual variations between a high of 5,866 and a low of 1,866. Stitt,(2) in 1907, who differentiated dengue from influenza and other acute infections by means of leukocyte counts, observed that a progressive leukopenia occurred in dengue and that the total counts varied between 5,280 and 1,450. From differential counts made at different periods of the disease, he found that marked variations occurred, there being at first a large increase in small lymphocytes succeeded by a greater increase in large lymphocytes and finally by an increase in large mononuclear cells.

Ashburn and Craig,(3) in 1907, discussed the leukopenia of dengue and presented results obtained by Vedder, who made the following remarks concerning his work:

> The polymorphonuclear leukocytes are greatly decreased and the small lymphocytes are greatly increased, while the large lymphocytes are moderately increased during the latter days of the illness. These changes are to a greater or less degree, characteristic of the disease throughout its duration. The decrease in polymorphonuclears and the increase in small lymphocytes takes place almost at once, being very noticeable on the second day of illness.

From these and other observations on experimental cases, Ashburn and Craig concluded that—

> Dengue is characterized by a leukopenia and in the vast majority of instances by a decrease in the polymorphonuclear leukocytes and a marked

increase in the small lymphocytes; the increase in the small lymphocytes is constant throughout the disease.

Many later workers have commented on the leukopenia of dengue, and several studies have been made of experimentally induced infections. One of the most important of these studies was made by Siler, Hall, and Hitchens(4) (1926) who analyzed one hundred fifty-four leukocyte counts of blood specimens from forty-eight experimental cases of dengue, or an average of over three counts per case. Their conclusions were as follows:

> The leukocyte count is not a dependable early symptom, as counts taken within a few hours of onset show great variation and average about normal. With the progress of the disease the total leukocyte count is reduced, the mean for the first four days becoming progressively less. However, 25 per cent of our cases failed to show a leukopenia at any time during the course of the disease.
>
> The so-called "reversal" in the differential count, the reduction of the percentage of polynuclear neutrophiles and corresponding increase of lymphocytes, is shown to be a function of the total count, both in normal and in dengue cases. Dengue cases showing normal total counts do not show the differential change. The differential count of the white cells is thus seen to be of little diagnostic value in comparison with the total count.
>
> The degree of leukopenia appears to be independent of the temperature at the time the count was made, and also of the severity of the disease as judged by the highest temperature reached during the attack, or by the duration of fever.

In 1929 there appeared a report by Joannides(5) concerning the morphologic alterations in the leukocytes during dengue, which is of considerable interest in connection with the present study. During the dengue pandemic in Athens in 1928, Joannides examined the blood of patients on different days of their illness, using smear preparations fixed with alcohol and stained by Giemsa's method. He noted especially profound morphological alterations both in the large mononuclears and the neutrophilic polymorphonuclear cells.

PRESENT INVESTIGATION

The following analysis of the leukocyte changes associated with dengue is based on total and differential leukocyte counts made on eighty-one experimental American volunteers. Throughout the various investigations of dengue carried on by the board, it was a routine procedure to examine the blood of each volunteer daily, in order to make the diagnosis in the experimental infections more certain. In practically all cases the counts were made at 6.30 a. m., before breakfast, and the afternoon counts were usually made between 4 and 4.30 p. m.

THE LEUKOCYTES OF NORMAL INDIVIDUALS

Volunteers admitted to the hospital for the various dengue experiments were generally observed for a preliminary period of eight or nine days, in order to obviate the possibility of outside infections. In many instances leukocyte counts were made daily throughout the preliminary period of normal observation. The results of these observations, which are summarized in Table 15 and fig. 21, furnish normal figures for comparison with those obtained during and following dengue fever.

THE LEUKOCYTES DURING SEVEN DAYS PRECEDING THE ONSET OF DENGUE FEVER

As shown in Table 15 and fig. 21, the average leukocyte counts during a period of seven days prior to the onset of dengue

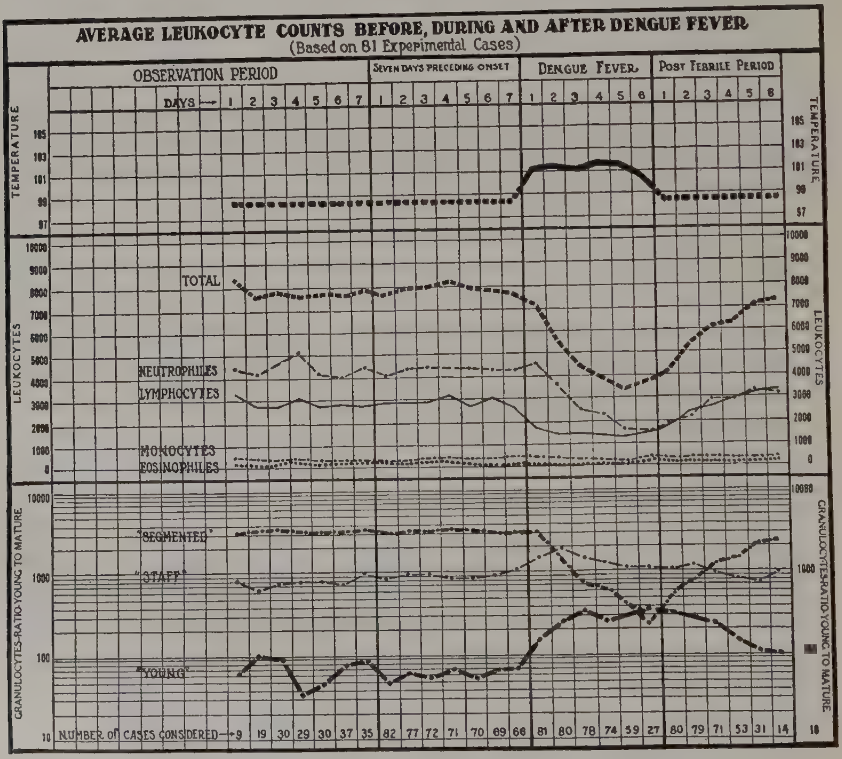

Fig. 21. Average leukocyte counts before, during, and after dengue fever.

fever were essentially similar to those noted in the normal observation period, except that on the day preceding fever the average figures indicate an alteration in the proportion of immature to mature forms of the granulocytes.

THE LEUKOCYTES DURING DIFFERENT DAYS OF EXPERIMENTALLY INDUCED DENGUE FEVER; TOTAL NUMBERS OF LEUKOCYTES

In the cases considered in Table 15 and fig. 21, the average total leukocyte counts were normal during the first day of the fever. However, as will be seen by reference to the charts in the Appendix, in some cases there was a slight leukopenia at the beginning of the initial rise in temperature, while in others there was a definite increase of short duration in leukocytes. In general, the leukopenia began on the second day of fever and progressed daily, reaching an average low point of 3,386 in about five days after onset. Thereafter the return of the leukocytes to normal was gradual, and required a period of from three to five days after the temperature had become normal.

Leukopenia occurred in every case at some time during the febrile period, with two exceptions. One of these patients had furunculosis and the other acute bronchitis. However, in the former there was a relative decrease in leukocytes, and in the latter definite leukopenia occurred during the post-febrile period.

The results shown in Table 16 indicate the proportion of cases in which one may expect to find leukopenia both during and following fever. Although all cases had leukopenia at some time during the fever, it will be noted that this sign was found more constantly toward the close of the febrile period and was constant for a few days following the last day of fever. The results emphasize the fact that in any case suspected as dengue, the blood should be examined on several successive days before it can be concluded that leukopenia does not exist.

The relationship between the fevers of different durations and the average total leukocyte counts is indicated in Tables 17, 18, 19, 20, and 21, and text figs. 22, 23, 24, 25, and 26.

It will be noted that in cases with fever lasting only two days, the leukopenia usually began on the second day. The average low point reached was 4,437 cells on the second day after the fever had subsided; and the leukopenia persisted in the average throughout the six-day postfebrile period. The total period of leukopenia in these two-day fever cases was about seven days.

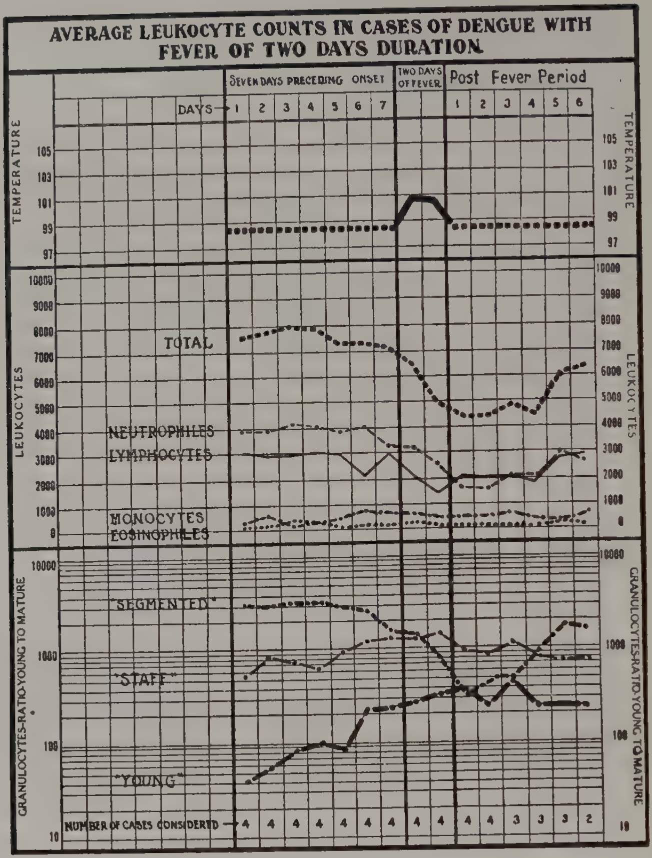

FIG. 22. Average leukocyte counts in cases of dengue with fever of two days duration.

In a small group of patients who had fever for three days, the average curve showed leukopenia on the second and third days, reaching an average low point of 4,375 on the first day of normal temperature, with a return to normal on the third day after subsidence of the fever. The average total period of leukopenia was four days.

In the cases with fever lasting four days, the average leukocyte curve showed a progressive fall beginning on the second day of fever and reaching a low point of about 3,520 on the first day of normal temperature; after which the counts rose daily until they were normal about the fourth day following the fever. The total average period of leukopenia in this four-day-fever group was about six days.

TABLE 15.—*Average leukocyte counts before, during, and after dengue fever.*

NORMAL OBSERVATION.

Days.	Cases considered.	Temperature.	Leukocyte counts.								
			Pulse.	Total.	Granulocytes.					Lymphocytes.	Monocytes.
					Neutrophilic.				Eosinophilic.		
					Segmented.	Staff.	Young.	Total.			
1	9	98.4	84	8,255	3,016	840	61	4,292	247	3,222	433
2	19	98.1	81	7,681	3,494	650	112	4,108	126	2,843	364
3	30	98.1	79	7,850	3,686	731	94	4,768	218	2,827	309
4	29	97.8	80	7,634	3,262	788	35	5,136	231	3,029	306
5	30	98.1	80	7,773	3,286	793	47	4,133	216	2,855	336
6	37	97.9	77	7,655	3,263	740	76	4,068	238	2,915	379
7	35	98.2	80	7,929	3,588	949	84	4,473	256	2,814	337
Average ----		98.1	80	7,825	3,371	784	72	4,425	219	2,929	352
SEVEN DAYS PRECEDING ONSET OF DENGUE.											
7	66	97.8	78	7,746	3,143	880	49	4,097	226	2,981	376
6	69	97.9	78	7,946	3,353	928	62	3,369	229	2,960	360
5	70	97.8	78	8,016	3,363	936	57	4,445	235	2,983	376
4	71	97.8	77	8,165	3,420	879	67	4,357	259	3,104	402
3	72	98.0	79	7,937	3,417	859	54	4,342	212	2,854	375
2	77	97.8	79	7,821	3,169	911	65	4,196	211	3,007	366
1	82	97.8	79	7,711	3,146	1,116	70	4,267	237	2,779	398
Average ----		97.9	78	7,906	3,287	930	61	4,282	231	2,953	379
FEVER PERIOD.											
1	83	101.1	84	7,129	3,046	1,608	181	4,607	182	1,818	416
2	82	101.3	90	5,617	1,556	1,903	280	3,661	64	1,475	372
3	80	101.2	88	4,569	787	1,602	301	2,663	58	1,537	340
4	77	101.5	87	4,035	629	1,392	283	2,322	53	1,369	271
5	62	101.4	86	3,386	425	1,127	299	1,873	58	1,345	217
6	29	100.6	86	3,800	374	1,162	351	1,887	58	1,588	287
Average ----		101.1	87	4,756	1,136	1,469	283	2,836	79	1,522	317
POSTFEVER PERIOD.											
1	80	98.0	75	4,314	554	1,051	321	1,949	113	1,959	379
2	79	97.8	76	5,469	787	1,119	261	2,369	182	2,454	407
3	71	97.8	77	6,195	1,274	963	243	2,803	133	2,660	461
4	53	97.7	77	6,441	1,499	809	155	2,848	142	2,918	415
5	31	98.0	75	7,103	2,191	775	128	3,343	182	3,194	426
6	14	97.8	79	7,219	2,273	939	116	3,165	207	3,148	415
Average ----		97.9	76	6,123	1,429	944	204	2,746	159	2,722	417

TABLE 16.—*Leukopenia on the different days of dengue.*[a]

DENGUE FEVER.

Day.	Cases considered.	Leukopenia.		No leukopenia.	
		Number.	Per cent.	Number.	Per cent.
1	82	24	29.27	58	70.73
2	81	53	65.43	28	34.57
3	79	69	87.34	10	12.66
4	75	71	94.68	4	5.34
5	60	57	95.00	3	5.00
6	28	26	92.85	2	7.15
FOLLOWING RETURN OF TEMPERATURE TO NORMAL.					
1	80	72	90.00	8	10.00
2	79	58	73.42	21	26.58
3	71	31	43.66	40	56.34
4	53	16	30.19	37	69.81
5	32	9	28.12	23	71.88
6	14	3	21.43	11	78.57

[a] Leukopenia occurred in all the cases at some time during the fever.

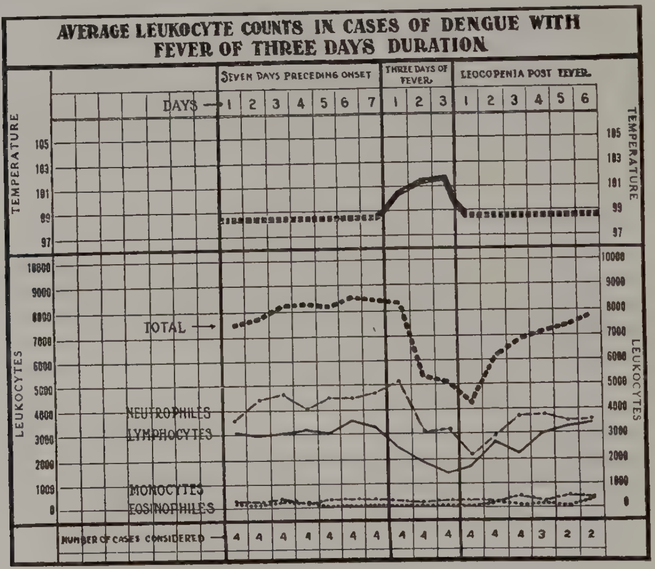

FIG. 23. Average leukocyte counts in cases of dengue with fever of three days duration.

TABLE 17.—*Average leukocyte counts in cases of dengue with fever of two days duration.*

SEVEN DAYS PRECEDING FEVER.

Days.	Cases considered.	Temperature.	Leukocyte counts.							
			Total.	Granulocytes.					Lymphocytes.	Monocytes.
				Neutrophilic.				Eosino-philic.		
				Seg-mented.	Staff.	Young.	Total.			
7	4	(a)	7,662	3,229	553	40	3,997	272	3,035	483
6	4	(a)	7,800	3,113	806	55	3,974	297	2,947	578
5	4	(a)	7,962	3,452	730	81	4,263	402	2,966	299
4	4	(a)	7,825	3,393	638	97	4,127	345	3,082	328
3	4	(a)	7,325	2,939	936	83	3,819	164	2,929	467
2	4	(a)	7,312	2,626	1,238	223	4,089	233	2,239	720
1	4	(a)	7,175	1,800	1,259	242	3,301	186	2,952	663
(Maximum)[b]			9,300	4,092	2,123	616	5,544	1,215	4,071	1,078
(Minimum)[b]			6,700	1,035	365	75	1,725	69	1,001	79
Average			7,580	2,936	880	117	3,939	271	2,879	505
FEVER PERIOD.										
1	4	100.9	6,539	1,664	1,256	277	3,198	313	2,129	602
2	4	100.6	4,950	799	1,540	342	2,681	164	1,526	520
(Maximum)[b]		102.2	7,200	2,849	2,548	476	4,134	486	2,592	1,248
(Minimum)[b]		97.2	4,300	44	616	208	924	0	583	301
Average		100.8	5,744	1,231	1,398	309	2,939	238	1,827	561
POSTFEVER PERIOD.										
1	4	(a)	4,462	326	932	364	1,623	116	2,137	540
2	4	(a)	4,437	443	872	250	1,565	179	2,069	506
3	3	(a)	4,850	508	1,014	485	2,008	94	2,070	611
4	3	(a)	4,550	984	797	238	2,020	158	1,856	418
5	3	(a)	6,150	1,706	727	225	2,958	205	2,775	404
6	2	(a)	6,375	1,632	765	229	2,626	192	2,818	510
(Maximum)[b]			7,650	2,448	1,336	627	3,901	307	3,900	969
(Minimum)[b]			2,900	153	434	0	1,274	58	841	186
Average			5,137	933	851	299	2,133	157	2,287	498

[a] Normal.

[b] Not averages.

TABLE 18.—*Average leukocyte counts in cases of dengue with fever of three days duration.*

SEVEN DAYS PRECEDING FEVER.

Days.	Cases considered.	Temperature.	Leukocyte counts.				
			Total.	Granulocytes.		Lymphocytes.	Monocytes.
				Neutrophilic.	Eosinophilic.		
7	4	([a])	7,525	3,720	238	3,104	352
6	4	([a])	7,862	4,403	175	2,910	348
5	4	([a])	8,287	4,716	245	3,068	394
4	4	([a])	8,350	4,018	275	3,316	190
3	4	([a])	8,200	4,548	61	3,023	245
2	4	([a])	8,562	4,468	75	3,686	395
1	4	([a])	8,475	4,782	134	3,391	368
(Maximum)[c]			12,100	7,744	605	5,568	968
(Minimum)[c]			6,450	2,819	0	2,092	161
Average			8,179	4,379	172	3,214	327
FEVER PERIOD.							
1	4	100.7	8,237	5,243	51	2,570	329
2	4	101.3	5,375	3,072	31	1,989	268
3	4	101.6	5,025	3,150	25	1,545	303
(Maximum)[c]		104	11,600	9,512	137	3,699	472
(Minimum)[c]		99	3,150	1,549	0	1,116	126
Average		101.2	6,212	3,822	36	2,035	100
POSTFEVER PERIOD.							
1	4	([a])	4,375	2,202	34	1,821	267
2	4	([a])	6,262	2,980	255	2,739	232
3	4	([a])	6,812	3,779	58	2,282	441
4	3	([a])	7,117	3,733	94	2,957	259
5	2	([a])	7,425	3,560	74	3,269	483
6	2	([a])	7,750	3,551	306	3,434	418
(Maximum)[c]			8,100	4,785	476	4,131	670
(Minimum)[c]			3,700	1,599	0	1,406	123
Average			6,624	6,301	137	2,750	350

[a] Normal.
[b] No Schiling counts made.
[c] Not averages.

TABLE 19.—*Average leukocyte counts in cases of dengue with fever of four days duration.*

SEVEN DAYS PROCEEDING FEVER.

Days.	Cases considered.	Temperature.	Leukocyte counts.							
			Total.	Granulocytes.					Lymphocytes.	Monocytes.
				Neutrophilic.				Eosinophilic.		
				Segmented.	Staff.	Young.	Total.			
7	12	(a)	8,196	3,424	839	22	4,295	291	3,285	326
6	12	(a)	8,229	3,575	877	69	4,506	195	3,091	384
5	12	(a)	8,400	3,670	738	67	4,475	248	2,304	428
4	12	(a)	8,829	3,904	743	71	4,666	284	3,245	506
3	12	(a)	7,950	3,427	748	46	4,157	218	3,088	374
2	13	(a)	8,373	3,339	811	93	4,222	271	3,301	514
1	14	(a)	7,557	3,197	982	73	4,197	202	2,670	464
(Maximum)[b]			12,300	5,600	3,198	369	7,749	1,069	4,669	1,674
(Minimum)[b]			5,900	649	268	0	3,127	0	1,485	0
Average			8,219	3,505	818	63	4,360	244	2,998	428
FEVER PERIOD.										
1	14	100.9	7,007	2,878	1,567	202	4,640	192	1,667	452
2	13	101	5,912	1,919	1,669	251	3,624	80	1,631	533
3	15	101.2	4,963	942	1,477	391	2,646	47	1,604	503
4	15	101.4	4,517	715	1,416	322	2,550	69	1,468	387
(Maximum)[b]		104	9,650	6,552	2,832	686	7,720	786	2,848	1,914
(Minimum)[b]		99.2	3,100	158	341	52	1,094	0	606	32
Average		101.1	5,600	1,613	1,532	291	3,385	97	1,592	469
POSTFEVER PERIOD.										
1	15	(a)	3,520	409	1,103	280	1,815	153	1,810	391
2	14	(a)	5,132	700	1,145	340	2,171	161	2,336	395
3	14	(a)	6,093	1,269	1,028	161	2,574	164	2,915	360
4	10	(a)	6,575	1,271	893	146	2,529	173	3,331	476
5	7	(a)	7,678	2,819	610	95	3,579	361	3,328	537
6	4	(a)	7,987	3,342	721	58	4,121	202	3,148	459
(Maximum)[b]			10,900	3,612	1,782	819	4,505	517	5,014	1,668
(Minimum)[b]			2,700	162	380	51	792	36	1,056	38
Average			6,164	1,634	917	180	2,798	202	2,811	436

[a] Normal.

[b] Not averages.

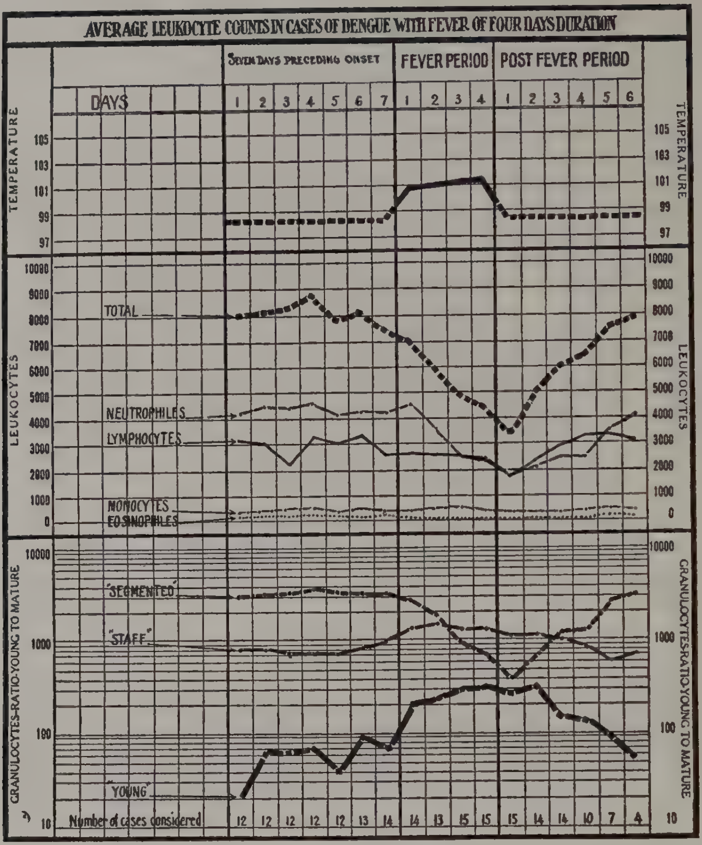

FIG. 24. Average leukocyte counts in cases of dengue with fever of four days duration.

The curve for the five-day-fever group also showed that leukopenia began on the second day, progressed daily, and reached a minimum point of 3,172 on the fifth day of fever. From this point the average counts increased, reaching normal four days later. The average duration of leukopenia in this group of cases was six days.

The cases with fever lasting six days showed a very similar average leukocyte curve during the period of fever, with the leukopenia persisting for two days afterward, or for a total of about seven days.

From a consideration of all these groups it may be stated that leukopenia was always apparent by the second day of fever; and that, although the lowest average counts in the groups of cases with short febrile periods were not quite so low, the

TABLE 20.—*Average leukocyte counts in cases of dengue with fever of five days duration.*

SEVEN DAYS PRECEDING FEVER.

Days.	Cases considered.	Temperature.	Leukocyte counts.							
			Total.	Granulocytes.					Lymphocytes.	Monocytes.
				Neutrophilic.				Eosinophilic.		
				Segmented.	Staff.	Young.	Total.			
7	22	([a])	8,261	3,346	965	79	4,410	261	3,132	410
6	25	([a])	7,880	3,399	847	65	4,211	211	3,009	332
5	25	([a])	7,830	3,309	812	19	4,209	243	2,970	387
4	26	([a])	8,010	3,558	803	58	4,324	251	2,900	404
3	27	([a])	8,191	3,552	922	48	4,578	231	2,940	411
2	29	([a])	7,595	3,190	807	48	4,137	227	2,868	337
1	31	([a])	7,931	3,410	1,091	59	4,352	305	2,680	457
(Maximum)[b]			11,150	5,555	4,263	334	8,251	930	5,329	1,069
(Minimum)[b]			5,950	307	298	0	1,836	0	1,540	0
Average			7,957	3,395	892	54	4,317	247	2,927	391
FEVER PERIOD.										
1	32	101.7	7,146	3,225	1,607	185	4,838	165	1,577	451
2	32	101.4	5,723	1,627	1,851	334	3,623	43	1,474	447
3	32	100.9	4,395	822	1,430	292	2,592	87	1,426	307
4	32	102	3,919	740	1,292	300	2,033	51	1,275	253
5	32	101.2	3,172	447	1,076	301	1,912	30	1,054	208
(Maximum)[b]		104.4	9,900	5,247	4,717	819	7,209	1,089	3,857	1,384
(Minimum)[b]		98.4	1,900	76	351	0	646	0	328	0
Average		101.4	4,871	1,372	1,451	282	2,999	75	1,361	333
POSTFEVER PERIOD.										
1	31	([a])	4,374	525	950	355	1,912	155	1,869	352
2	31	([a])	5,661	959	1,120	252	2,524	143	2,563	512
3	27	([a])	6,505	1,603	855	146	2,744	150	3,024	462
4	20	([a])	7,055	1,660	628	114	2,711	145	3,311	516
5	9	([a])	7,211	2,145	762	96	3,295	136	3,377	409
6	3	([a])	7,650	2,905	688	115	3,670	203	3,213	495
(Maximum)[b]			8,700	3,268	1,848	891	5,046	655	4,514	1,192
(Minimum)[b]			2,150	117	196	0	539	0	881	0
Average			6,409	1,633	834	179	2,809	155	2,893	458

[a] Normal.

[b] Not averages.

minimum was reached, as in the fevers of long duration, on the fourth or fifth day after onset; and that the duration of leukopenia in all groups was about four to seven days. This fact indicates that regardless of the duration of fever the decrease

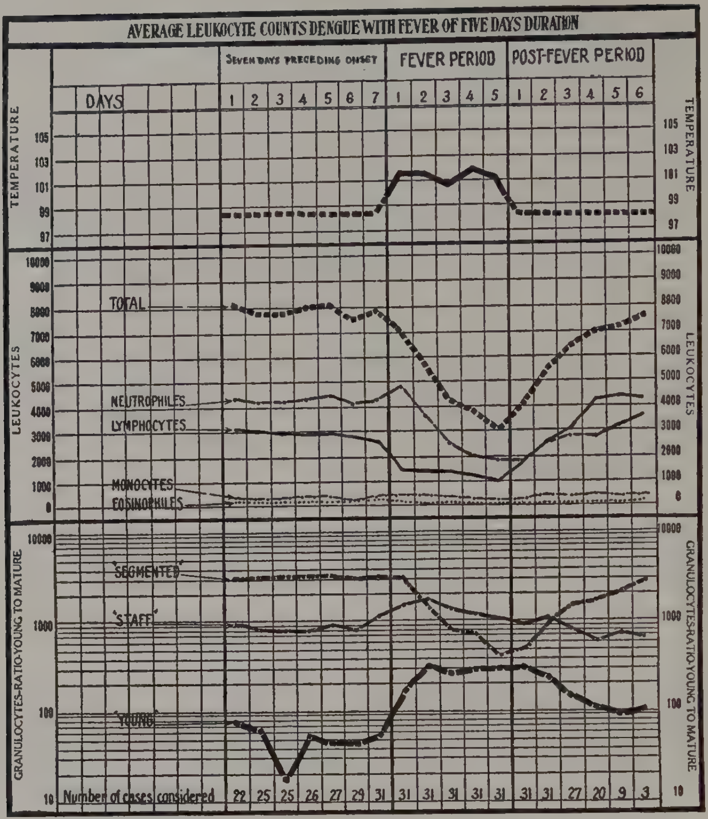

FIG. 25. Average leukocyte counts in cases of dengue with fever of five days duration.

in the number of leukocytes, once begun, progresses to a low point which occurs on the fourth, fifth, or sixth day, and that thereafter the number gradually increases and returns to normal. Apparently, differences in the length of the febrile period do not influence to any marked extent either the degree or duration of leukopenia.

THE LYMPHOCYTES

The average daily counts of the numbers of lymphocytes in the various groups of cases considered showed the following general trend (Table 15): During the periods of normal observation and incubation, the average number of cells varied between 2,827 and 3,222, with an average of about 2,900. On the day preceding onset of dengue the lymphocytes were slightly decreased, while on the first day of dengue fever the lymphocytes

TABLE 21.—*Average leukocyte counts in cases of dengue with fever of six days duration.*

Days.	Cases considered.	Temperature.	Leukocyte counts.							
			Total.	Granulocytes.					Lymphocytes.	Monocytes.
				Neutrophilic.				Eosinophilic.		
				Segmented.	Staff.	Young.	Total.			
SEVEN DAYS PRECEDING FEVER.										
7	23	(a)	7,720	3,776	978	40	3,984	193	2,898	380
6	24	(a)	7,935	3,170	1,059	68	4,375	256	2,935	344
5	25	(a)	7,978	3,162	1,172	68	4,456	221	2,887	332
4	25	(a)	8,032	3,018	1,065	67	4,216	261	3,198	374
3	25	(a)	7,672	3,186	880	46	4,202	232	2,867	330
2	27	(a)	7,689	3,054	1,006	38	4,075	187	2,997	276
1	28	(a)	7,552	3,026	1,183	54	4,283	198	2,724	266
(Maximum)[b]			13,300	6,765	5,586	294	8,778	940	4,980	1,200
(Minimum)[b]			3,900	195	246	0	1,448	0	1,410	0
Average			7,797	3,199	1,049	54	4,227	221	2,929	329
FEVER PERIOD.										
1	27	101.4	7,181	2,840	1,574	118	4,622	173	2,061	315
2	27	102.0	5,605	1,452	2,219	218	3,924	74	1,241	275
3	27	101.0	4,656	715	1,839	213	2,724	27	1,632	280
4	27	101.2	3,852	450	1,448	223	1,913	51	1,404	206
5	27	101.5	3,509	390	1,208	274	1,825	91	1,278	232
6	27	100.6	3,713	227	1,224	339	1,902	52	1,486	276
(Maximum)[b]		104.6	9,800	5,947	4,588	1,512	7,278	749	3,952	1,228
(Minimum)[b]		98.6	2,100	28	370	0	798	0	380	0
Average			4,753	1,012	1,585	231	2,818	78	1,517	264
POSTFEVER PERIOD.										
1	27	(a)	4,837	656	1,218	307	2,193	105	2,159	344
2	26	(a)	5,854	1,051	1,291	204	2,607	171	2,564	392
3	23	(a)	6,717	1,716	955	183	2,908	188	3,008	432
4	17	(a)	6,906	2,127	919	124	3,246	140	3,133	409
5	10	(a)	7,050	2,095	1,001	97	3,321	134	3,222	298
6	3	(a)	6,333	1,212	1,584	63	2,859	132	3,127	194
(Maximum)[b]			8,800	5,104	2,660	657	6,160	602	4,917	1,341
(Minimum)[b]			2,150	43	441	0	530	0	957	0
Average			6,283	1,809	1,161	163	2,856	145	2,869	345

[a] Normal.

[b] Not averages.

were usually decreased about 1,000 cells. Some decrease was maintained throughout the febrile period with an average low point of 1,522 cells, or approximately one-half the average nor-

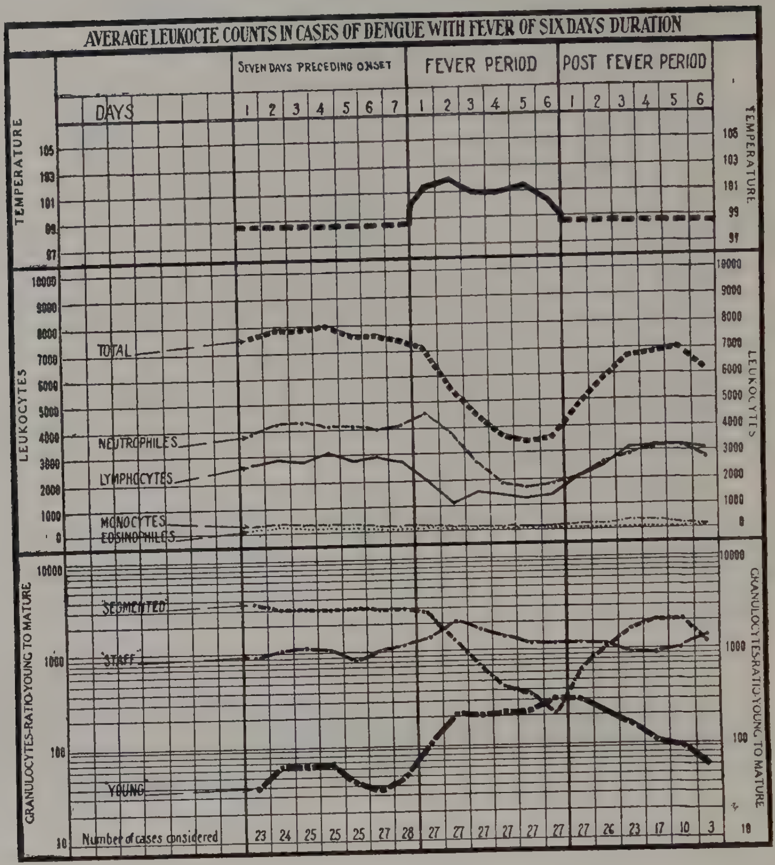

FIG. 26. Average leukocyte counts in cases of dengue with fever of six days duration.

mal number. Although the average reduction in the number of lymphocytes in the eighty-one cases considered extended over a period of nine days, the charts of the individual fever groups show that this period might be as short as six days, but as a general rule the lymphocytes returned to normal before the granulocytes.

THE MONOCYTES

The average daily counts of monocytes during the period of normal observation and incubation varied from a minimum of 306 to a maximum of 433, with an average of 365 (Table 15). While no significant changes were noted in the total numbers of the monocytes during dengue, a large vacuolated mononuclear cell, morphologically similar to the so-called Turk "irritation cell," made its appearance in the majority of cases either during the last days of fever or at some time in the period of convalescence. This type of cell has been noted by various observers in other febrile conditions including Tsutsugamuchi disease, in which Kawamura(6) observed that "Turk" cells usually appeared late, or at the time when the eruption was fading. Although the finding of these cells was not considered as diagnostic, their occurrence came to be regarded as suggestive of dengue.

THE GRANULOCYTES (EOSINOPHILIC)

The average daily count of the eosinophiles before the onset of fever was 231. For the period of fever the average was 79 and in the postfebrile period it was 159 (Table 15). Because of the small numbers of the eosinophiles present, these changes may be of no significance, but it is interesting to note that the eosinophiles apparently follow the general downward trend of the other granulocytes.

THE GRANULOCYTES (BASOPHILIC)

The basophiles showed no noticeable change and were omitted from the charts.

THE GRANULOCYTES (NEUTROPHILIC)

The most significant diagnostic leukocytic reactions associated with dengue fever are the profound changes in the neutrophilic granulocytes. It will be noted that the average daily neutrophilic curves parallel those of the total leukocytes.

The average daily numbers of neutrophiles during the periods of normal observation and incubation varied from 3,369 to 5,136, with an average for both groups of about 4,300. During the first day of dengue fever the average showed a slight increase. This was followed on the second day of fever by a sharp decrease of about 1,000 cells. Thereafter, the decline was progressive until a low point below 2,000 was reached at some time between the fourth and sixth days following onset of dengue, regardless of the duration of the fever. Following this low point the numbers of neutrophiles slowly increased daily; but in a majority of the groups, and in the average of all groups, they were still slightly below normal even on the sixth day of convalescence.

In addition to a marked decrease in numbers during dengue, the mature neutrophilic granulocytes also showed morphologic evidence of degenerative changes. During the course of the disease, especially during the period of extreme leukopenia, it was not infrequently noted that the nuclei of these cells were markedly altered; in many cells they were vacuolated, irregular in outline, and poorly stained; in some they were fragmented, portions of nuclear material being found irregularly distributed in the cytoplasm. The cytoplasm of the cells often showed a marked loss of neutrophilic granules with some clumping of those remaining. The outlines of these cells were usually indefinite and appeared to fade into surrounding areas. Because of the marked destruction of the circulating granulocytes, one should expect to find some compensating reaction on the part of the blood-forming organs. This reaction is shown by a study of the granulocytes, which were arbitrarily divided into three groups as follows:

"Segmented" neutrophiles.—These cells, which are considered to be the mature forms of the granulocytes, are characterized by a multilobed nucleus segmented or divided into two or more lobes connected by a thread of nuclear material.

"Staff" or "band" form neutrophiles.—These cells are considered to be younger than the segmented forms. They have a nonsegmented nucleus which is narrow and usually curved. The cytoplasmic structure is similar to that of the fully matured cells.

"Young" granulocytes or metamyelocytes.—These cells are considered to be more immature than the "staff" form and slightly older than the myelocytes. The nucleus is usually rounded in outline and often shows slight indentations. The cytoplasmic structures are similar to those of the mature cells.

It is apparent from a study of the charts and tables, that the leukopenia of dengue was due largely to a destruction of the mature granulocytes. These cells actually decreased from a normal level of approximately 3,000 to the alarmingly low figure of about 300 cells. Fortunately, this loss of mature granulocytes was partially compensated for by an immediate increase in the immature forms, which doubled or tripled in numbers during the febrile period.

The mature, or "segmented," forms usually remained at the normal level of about 3,000 cells until the second day of the fever, when there was a fall of about 50 per cent, following which the numbers of cells continued to decrease to a low point of about 300 to 400 on the fifth or the sixth day following the onset. After this low point was reached the numbers of mature granulocytes gradually increased toward normal during convalescence, but in the average of all cases and in some of the groups the normal level was not reached even on the sixth day following normal temperature.

In the majority of the fever groups, the "staff," or immature, forms increased to a point above the upper normal limit of 1,000 cells on the day preceding the febrile period, but the rise shown by the average of all cases was insignificant. In general, it may be stated that these cells doubled in numbers on the first day of fever, which no doubt accounted for the slight elevation observed in the total neutrophilic count on the first day. On the second day of fever the average number of staff forms reached a peak of about 1,900 cells, which also sustained the total count to a level only slightly below normal. From this high point the staff forms remained increased in number during fever and for three or four days following the return of temperature to normal.

The "young"-form granulocytes, the upper normal limit of which was considered to be about 100 cells, showed a sharp rise on the first day of fever and were more than doubled in numbers by the second day. At the period of greatest depression of the mature forms the young granulocytes were still increased in numbers and in the majority of the four groups they later became more numerous than the former. The highest point was usually reached between the fifth and sixth days of fever; following which they remained above normal for about three more days in the different fever groups, while the average of all cases showed an increase in these cells during the entire six days of the postfebrile period.

From a consideration of the changes in the proportions of the three types of granulocytes, it may be stated that an increase in the number of immature cells, associated with a normal number or a slight decrease in the number of mature forms, during the first day of a febrile condition clinically resembling dengue, is a valuable diagnostic sign. In many individual cases this reaction appeared on the day preceding the onset and made it possible to forecast a diagnosis of dengue in these experimental cases.

In cases with fever, leukopenia, and subjective symptoms of dengue the increase in immature granulocytes above the numbers of mature segmented forms is considered to be the most important confirmatory diagnostic sign of the disease.

SUMMARY AND CONCLUSIONS

1. In the cases of experimental dengue included in this study, the leukopenia usually began on the second day, became progressively more extreme, reaching a low point on the fourth, fifth, or sixth day after the onset, following which the leukocytes gradually returned to normal on the third or fourth day of convalescence.
2. The leukopenia was mainly due to a decrease in both the lymphocytes and mature neutrophilic granulocytes.
3. The numbers of lymphocytes returned to normal levels before the granulocytes, although both were decreased for several days during convalescence.
4. The marked degenerative changes that were observed in the cell structures, particularly in the mature granulocytes, indicate that the leukopenia of dengue is due to a destruction of these cells. This suggests the possibility that the virus may multiply within the leukocyte, causing the death of the cell.
5. Coincident with the leukopenia there occurred an enormous increase in immature granulocytes. This sharp rise in the number of younger cells at the time of the decrease in the older forms, or the so-called "shift to the left," was observed to be a constant reaction. In the early diagnosis this shift in the proportion of the immature granulocytes, together with the leukopenia, constituted the most reliable single diagnostic sign of dengue fever.

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DENGUE IMMUNITY

There is an abundance of epidemiological and clinical information bearing on the subject of dengue immunity, but relatively few experimental studies have been reported. Therefore, many questions remain to be answered concerning the mechanism, degree, and duration of this immunity. From observations concerning the high percentage of individuals attacked during the first introduction of dengue into various localities, and even in endemic areas during epidemic or pandemic waves of the disease, it seems probable that few if any human beings possess natural immunity to the infection. Outbreaks have been reported by Sandwith,(1) Godding,(2) Cleland, Bradley, and McDonald,(3) Legendre,(4) and others, in which from 75 to 100 per cent of entire populations have been infected within a limited period of time. A recent example is the explosive outbreak in Greece during 1927–1928, in which, according to a report by the League of Nations,(5) 239,000 cases occurred in Athens during the first eight months of 1928.

While adult natives living in endemic localities rarely become ill with the disease during inter-epidemic periods, there is no conclusive evidence to indicate that natural immunity to dengue exists in any group or race of people. The apparent racial immunity of certain peoples living in the Tropics is, no doubt, acquired as a consequence of previous attacks. According to Hirsch,(6) (1883) it was observed by Dickson that the only persons who escaped infection during the Charleston outbreak of 1850 were those who had previously been attacked in 1828. A most significant observation is that of Bonne,(7) (1918) in Paramaribo, who noted that Europeans admitted to his hospital developed dengue within a week or two; while no case occurred among one hundred Creole nurses and other attendants. However, he observed that the mulatto children were not immune. Other interesting observations have been made in the Fiji Islands. Skottowe(8) (1890) reported that during 1885, when dengue was probably first introduced, the native Fiji Islanders suffered more severely than the Melanesians. Later, during an epidemic which occurred there in 1906, Montague(9) observed that the old residents who had had the disease in 1885 escaped in almost every instance, and that in the intermission, the island had been particularly free from dengue. During the recent epidemic in Greece it was noted by Copanaris(10) (1928) that persons who had had the disease in 1927 escaped in 1928. It was remarked that "presumably some immunity had been produced and remained." Blanc and Caminopetros(11) (1929) made similar observations concerning the immunity of individuals who had dengue during the 1927 outbreak in Greece, and stated that, in their opinion, dengue confers a high degree of immunity which is of considerable duration.

Adult natives living in endemic areas of the Philippines are also comparatively resistant to dengue, as is indicated both by the observations of local physicians and by a comparison of the low dengue rates for Philippine Scout soldiers who have spent most of their lives in the infested areas, with the relatively high rates for white American troops stationed for short periods in the same localities. This fact was discussed by Siler, Hall, and Hitchens,(12) who made the following observations:

> In an area of endemicity it is likely that native children suffer their first attack of dengue early in life, and that the degree of immunity exhibited later in life is the result of acquired immunity constantly reënforced by frequent bites of infective mosquitoes. In every epidemic season a certain number of native people have clinical dengue. These may be persons who develop immunity with difficulty or who return to the susceptible state rapidly, or who have been removed from infective mosquitoes for a considerable period.

The fact that many adult natives of endemic areas are immune is also indicated by the results of transmission experiments. Ashburn and Craig(13) 1907, after the inoculation of fourteen volunteers at Fort William McKinley, observed that among seven men who had not previously been exposed, only one immune was found; while among seven individuals who had passed through an epidemic, two were completely immune, three were partially immune, and in one the result was doubtful. Siler, Hall, and Hitchens,(12) during their transmission experiments, were able to infect all but seven of sixty American volunteers inoculated with infective blood or bitten by infective mosquitoes. All but one of the seven immunes had been in the Philippines for periods of from three to nineteen months. However, in their experiments with thirty-two Philippine Scout soldiers from Fort William McKinley it was found that 100 per cent were immune. Schule(14) 1928 called attention to the fact that certain volunteers inoculated with dengue virus were immune and that all of these immunes had either been residents of the Philippines for a considerable period or had come from other localities where dengue was prevalent.

Somewhat similar results were obtained during the investigations conducted by the present board. Among eighty-seven American volunteers who were bitten by mosquitoes of infective lots only 4, or 4.6 per cent, failed to develop dengue. These four immunes had been in the Philippines for periods of three, six, six, and eight months, respectively, before they were tested. Similar experiments with Philippine Scouts stationed in or near Manila gave strikingly different results which are described below.

PART I. OBSERVATIONS CONCERNING THE IMMUNITY OF NATIVES OF THE PHILIPPINE ISLANDS

(A) ATTEMPTS TO PRODUCE DENGUE IN ADULT FILIPINOS LIVING IN AREAS IN WHICH THE DISEASE IS ENDEMIC

Among fifteen Philippine Scout soldiers, each inoculated with blood from experimental cases of dengue, and eighteen others bitten by infective lots of *Aëdes*, only two showed suspicious but unproved signs of infection; while thirty-one, or 93.9 per cent, were immune. In each experiment the soldiers were observed daily for evidence of infection, the temperature reactions were recorded and leukocyte counts were made each morning, in order to detect even slight changes which might indicate infection in individuals incompletely immune.

Experiment 1.—January 12, 1929. Infective blood, which had been collected in citrate from an experimental case of dengue (A.V. 1–H), thirty-six hours after onset of symptoms, and kept in the ice box for twenty-four hours, was inoculated, in 0.025 cubic centimeter amounts, into each of ten Philippine Scout soldiers stationed at Fort William McKinley. During subsequent observation none of these men developed symptoms recognizable as dengue. The records of their daily temperature reactions and leukocyte counts are shown below.

Filipino volunteer 1.—Pilorin, D., Serial No. 673082, Ilocano, born in Ilocos Sur, age 22, service in Army four years at Fort William McKinley. Has never been sick except for "chills and fever" in 1928. "Never had dengue or 'trankaso,' or any symptoms suggesting dengue." The highest temperature recorded for this man was 99° F. which occurred January 17, 1928, the fifth day after inoculation. The only leukocyte count low enough to indicate a mild leukopenia was 6,150 January 16, the fourth day after inoculation. These observations were not considered to be of diagnostic significance.

Filipino volunteer 2.—Dona, Alejandro, Serial No. R–310178, Ilocano, born in Ilocos Sur, age 27, service in Army eleven years at Fort William McKinley. Had "chills and fever" when a child, but has no knowledge of any attacks of "trankaso" or dengue. After inoculation the only elevation of temperature noted was 99 and 99.8° F. on the eighth day, January 20; the leukocyte counts January 16, 21, and 23 were 5,200, 6,250, and 6,100, respectively.

Filipino volunteer 3.—Robio, Catalino, Serial No. R–323307, Ilocano, born in Ilocos Norte, age 28, service in Army eleven years, including one

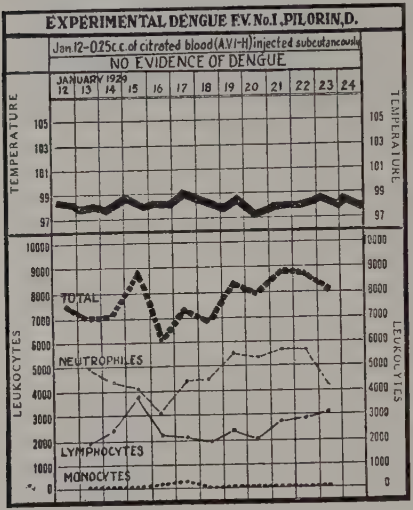

FIG. 27. Experimental dengue in Filipino volunteer 1.

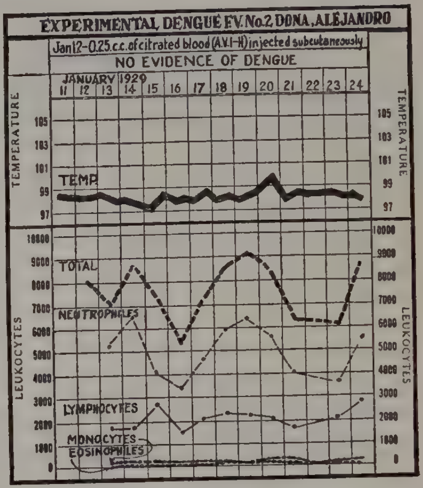

FIG. 28. Experimental dengue in Filipino volunteer 2.

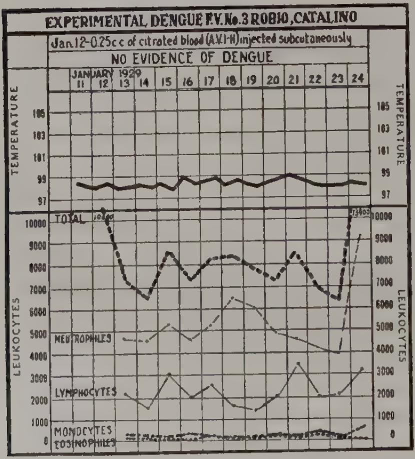

FIG. 29. Experimental dengue in Filipino volunteer 3.

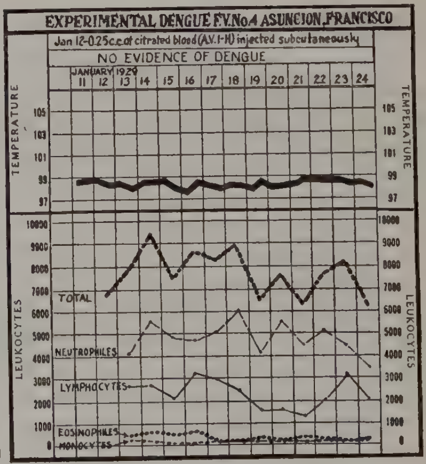

FIG. 30. Experimental dengue in Filipino volunteer 4.

year in Batangas and nine years in Fort William McKinley. Has never been sick. After inoculation the temperature was normal for twelve days and there was no significant reduction in leukocytes, with the possible exception of counts of 6,500 January 14 and 6,250 January 23, which were not considered diagnostic.

Filipino volunteer 4.—Asuncion, Francisco, Sgt., Serial No. 6736039, Ilocano, born in Abra, age 24, service in Army four years at Fort William McKinley. Had fever during childhood but has not been ill since he became grown. After inoculation his temperature was normal for twelve days. The leukocyte count was 6,500 January 19, 6,350 January 21, and 6,150 January 24. There was no evidence of dengue.

Filipino volunteer 5.—Laureta, Agustin, Serial No. R–321311, Ilocano, born in Abra, age 32, ten years service in Army at Fort William McKinley. "Never sick during childhood." Had "trankaso" in 1919 but "never had dengue." After inoculation with dengue blood the temperature remained normal. The leukocyte count was 13,000 on the second day, 6,600 on the sixth, and 5,700 on the eleventh. There was no evidence of dengue.

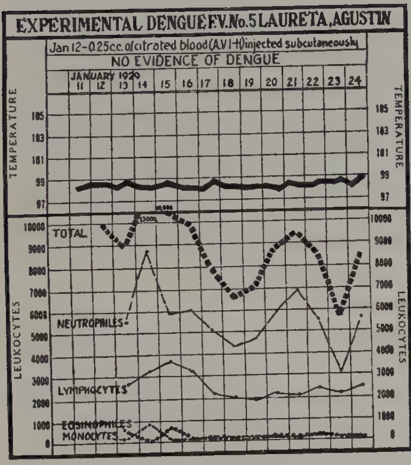

FIG. 31. Experimental dengue in Filipino volunteer 5.

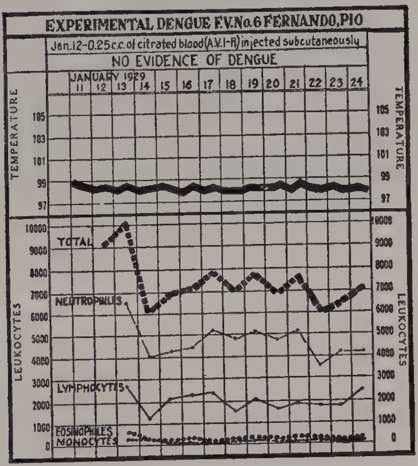

FIG. 32. Experimental dengue in Filipino volunteer 6.

Filipino volunteer 6.—Fernando, Pio, Cpl., Serial No. R–310605, Ilocano, born in Ilocos Sur, age 40, service in Army 21⅚ years (Lanao, 1913–1916; Camp McGrath, 1916–1919; Fort William McKinley, 1919–1929). Had fever during childhood, also malaria in 1915. Never had "trankaso" or dengue. After inoculation the temperature remained normal. The leukocyte count was 6,050 on the second day, 6,100 on the tenth day, and 6,500 on the eleventh day. There was no evidence of dengue.

Filipino volunteer 7.—Fontanilla, Jose, Serial No. 6736088, Ilocano, born in Ilocos Sur, age 24, service in Army four years at Fort William McKinley. Never sick during childhood, but had "trankaso" in 1918. After inoculation the temperature and total leukocyte counts were normal or slightly elevated for twelve days. There was no indication of dengue.

Filipino volunteer 8.—Balosan, Domingo, Serial No. R–321024, Ilocano, born in Abra, age 33, service in Army ten years at Fort William McKinley. Had "fever with aching of eyes and joints" during childhood and had "fever in 1918." After inoculation the temperature remained normal for twelve days and the lowest leukocyte count obtained was 5,450 on the fourth day. There was no evidence of dengue.

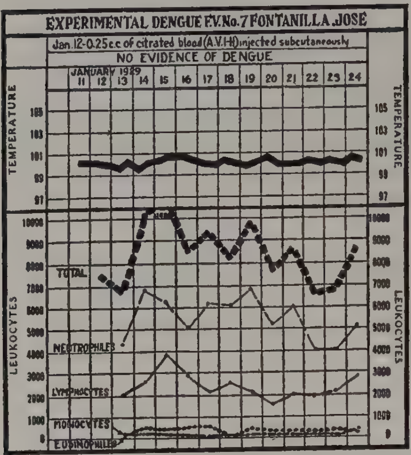

FIG. 33. Experimental dengue in Filipino volunteer 7.

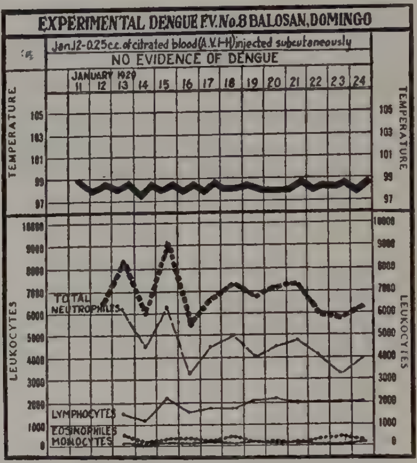

FIG. 34. Experimental dengue in Filipino volunteer 8.

Filipino volunteer 9.—Reyno, Pascual, soldier on duty at Fort William McKinley. No history of dengue. After inoculation the temperature remained normal and the leukocyte counts were decreased slightly after the fifth day. However, there was no evidence of dengue.

Filipino volunteer 10.—Abad, Jose, Serial No. 6610113, Tagalog, born in Nueva Ecija, age 27, lived in Cabanatuan, Nueva Ecija. Service in Army nine years at Fort William McKinley. Had "fever" when a child; also had "chills and fever" in 1917. This volunteer was injected with 0.5 cubic centimeter of infective dengue virus in February, 1925, by Siler, Hall, and Hitchens, but showed no reaction. After the present inoculation the temperature remained normal for twelve days, as did the total leukocyte count, except for a decrease to 5,850 on the fourth day. The leukocyte curve in this man was not considered significant of dengue, but was thought to be suggestive.

Experiment 2.—Filipino volunteer 12, Sales, Gervacio, Ilocano, born in Ilocos Norte, age 32, service in Army nine years, Fort Mills and United States Army Medical Department Research Board in Manila. Had "fever" during childhood, also "chills and fever" in 1924.

February 9, 1929, Sales was bitten by sixty-one infective *Aëdes ægypti*, lot 2, which had taken infective blood thirty-one days previously from an experimental case of dengue (A.V. 2–M) during the first day of the disease. These mosquitoes produced dengue in a susceptible American volunteer (A.V. 4–Y).

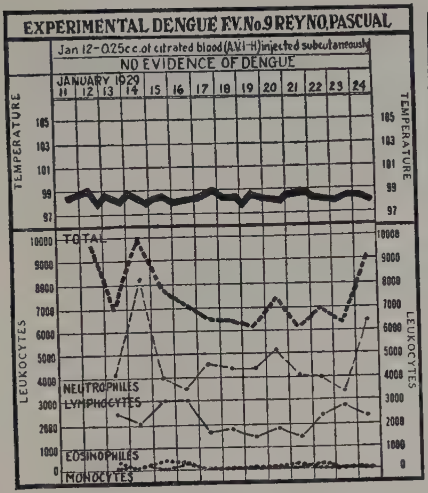

Fig. 35. Experimental dengue in Filipino volunteer 9.

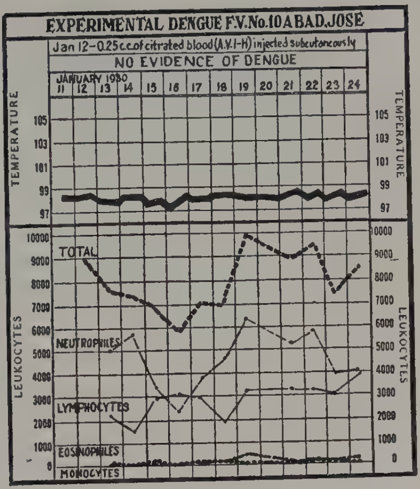

Fig. 36. Experimental dengue in Filipino volunteer 10.

After being bitten by the mosquitoes the temperature of F.V. 12–Sales, remained normal for twelve days, excepting a rise to 99.2° F. on the fifth day, at which time the leukocyte count was 6,850. There was no evidence of dengue. Note: This man was used later in experiment 5.

Experiment 3.—Filipino volunteer 11, Reyes, Bonifacio, Pangasinan, born in Pangasinan, age 31, service in Army seven years ten months, at United States Army Medical Department Research Board, Manila. During childhood had chicken pox, small pox, chills and fever; also had fever which he thinks was "dengue," with aching of eyes, bones, and joints, in 1919, 1924, and 1928.

February 9, 1929, was bitten by fifty-six infective *A. ægypti,* lot 3, which thirty days previously had taken blood from an experimental case of dengue (A.V. 1–H) during the first day of the disease. These mosquitoes transmitted dengue to a susceptible American volunteer (A.V. 3–A). After he had been bitten by the infective mosquitoes, Reyes's temperature and lymphocyte counts remained normal for at least twelve days. Note: This man was used later in experiment 6.

INOCULATION OF FILIPINOS WITH LARGE AMOUNTS OF DENGUE VIRUS IN AN ATTEMPT TO OVERCOME THEIR IMMUNITY

Experiment 4.—April 16, 1929. Blood collected in sodium citrate from an experimental case of dengue (A.V. 6–R) on the first day of the disease, was injected into three Filipino Scout soldiers as follows:

Filipino volunteer 17.—Yap, A., was injected subcutaneously with 5 cubic centimeters of the blood. This man, who was a private 1st class in the 12th Medical Regiment, was born in Leyte, age 31, had had six years service in the Army at Fort William McKinley. Previous medical history and physical examinations were negative. Three days after the inoculation of infective blood Yap was admitted to hospital complaining of headache and pain in his bones and joints. The temperature was 101.6° F. on this day. The total leukocyte counts on the fourth and fifth days were 5,250 and 5,350, respectively, and there was an indefinite rash over his body on the day of admission. Comment: Symptoms which might be ascribed to a mild attack of dengue were observed in this Filipino after the subcutaneous inoculation of 5 cubic centimeters of infective blood, and while the specific nature of the infection was not proved, it is believed that dengue occurred.

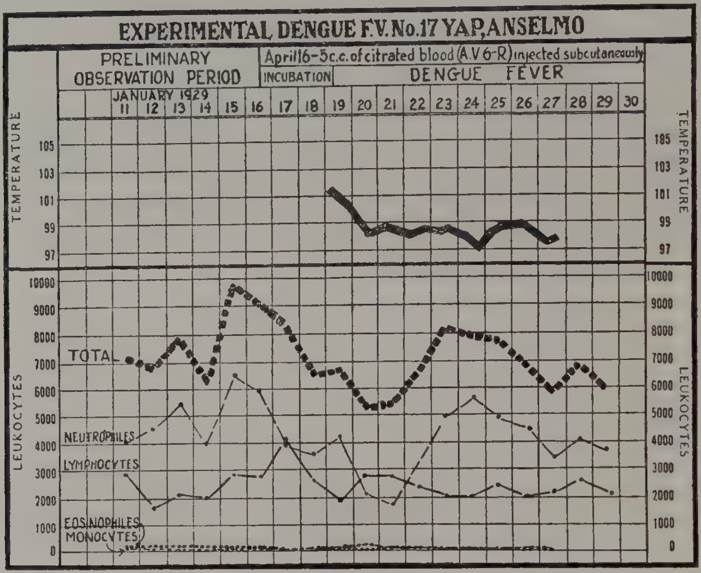

FIG. 37. Experimental dengue in Filipino volunteer 17.

Filipino volunteer 18.—Penas, Eleodoro, was injected intravenously with 0.5 cubic centimeter of the infective blood April 16. This man, who was a private in the 12th Medical Regiment, was born in Camarines Sur, age 26, service in Army five years at Fort William McKinley. Previous medical history and physical examination negative. Three days after the inoculation of blood, he was admitted to hospital complaining of headache, pains in eyes and joints. The temperature was elevated on the fourth, fifth, and sixth days. The only suggestive leukocyte counts were 5,250 on the seventh day and 6,050 on the eighth. Comment: This case, like the preceding one, presented symptoms which suggested the possibility of a mild dengue infection.

Filipino volunteer 19.—De los Santos, F., was inoculated subcutaneously with 8 cubic centimeters of the infected blood April 16. This man, who was a private in the 12th Medical Regiment, stationed at Fort William McKinley, was observed for a period of eleven days, during which he remained well and had normal temperature reactions and leukocyte counts. There was no indication of dengue.

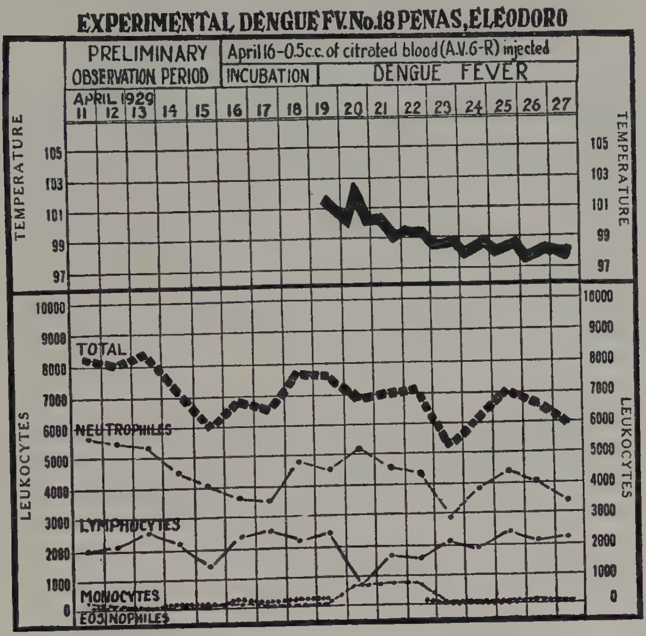

FIG. 38. Experimental dengue in Filipino volunteer 18.

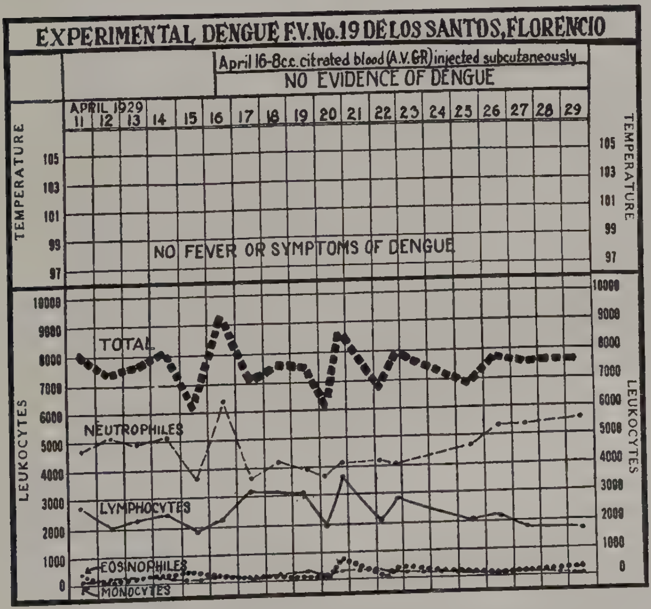

FIG. 39. Experimental dengue in Filipino volunteer 19.

AN ATTEMPT TO PRODUCE DENGUE IN AN IMMUNE VOLUNTEER BY REPEATED FEEDINGS OF DENGUE-INFECTED MOSQUITOES

Experiment 5.—Filipino volunteer 12, Sales, used for this experiment was a technician in the laboratory of the board. So far as he knew he had never had dengue, but stated that he had "fever" during childhood. Since 1928, two negative attempts were made to infect Sales with dengue; one (*a*) February 9, 1929, when he was bitten by sixty-one dengue-infected *A. ægypti* of lot 2, and the other (*b*) in 1929, when he was injected subcutaneously with citrated infective blood from an experimental case of dengue. The third attempt was made to overcome the resistance of this individual by first allowing two infective mosquitoes to feed on him and, later, introducing a larger dose of virus by allowing a large number of infected mosquitoes to feed.

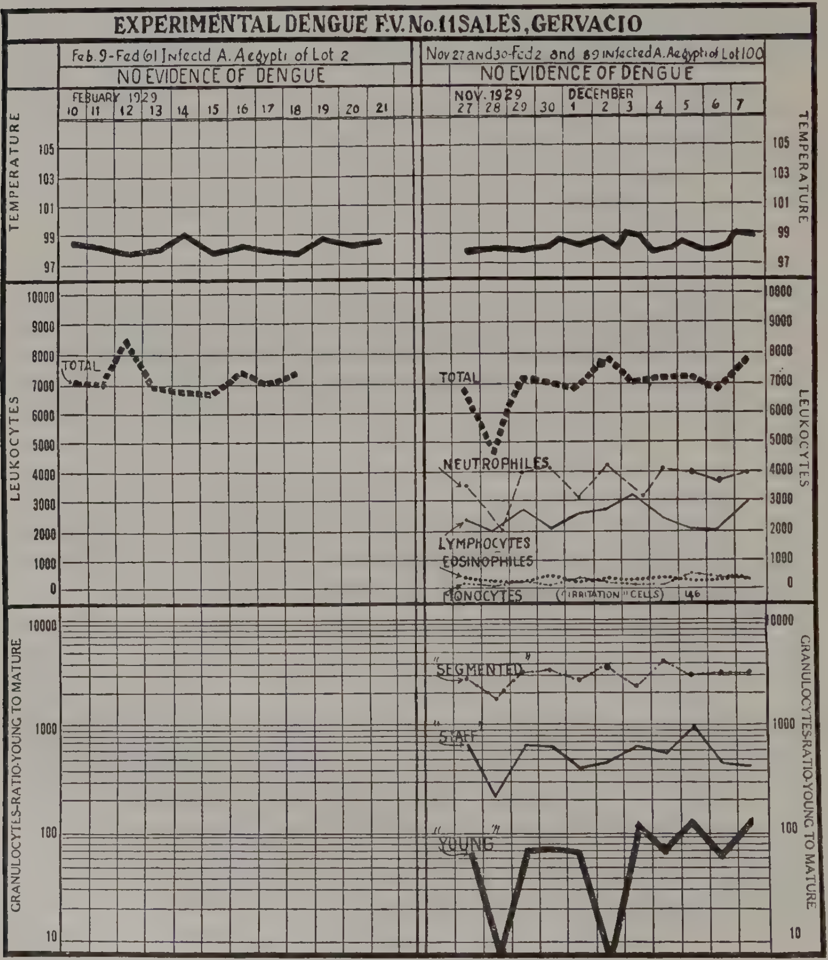

FIG. 40. Experimental dengue in Filipino volunteer 11.

November 27 Sales was bitten by two infective *A. ægypti*, lot 100, which had taken blood 27 days previously from an experimental case of dengue (A.V. 55–S), during the first day of the disease. November 30, he was bitten by eighty-nine infective *A. ægypti*, lot 100. Temperature and leukocyte count records made from November 27 to December 7, 1929, indicate that this man failed to develop even a mild infection. Comment: This adult Filipino was found to be immune to repeated doses of dengue virus.

AN ATTEMPT TO PRODUCE DENGUE IN AN IMMUNE FILIPINO VOLUNTEER BY REPEATED DAILY FEEDINGS OF INFECTIVE MOSQUITOES

Experiment 6.—Filipino volunteer 11. Reyes, selected for this experiment, was a laboratory technician who for six years had assisted in handling the mosquitoes used by the board in various dengue transmission experiments. Pvt. Reyes stated that he had had dengue fever several years previously.

Since 1928 two negative attempts were made to infect Reyes with dengue: (*a*) February 9, 1929, he was bitten by fifty-six dengue-infected *A. ægypti*, lot 3, and (*b*) later in 1929, he was inoculated subcutaneously with citrated infective blood from an experimental case of dengue. He remained well following both tests.

Present test November 19, 1929, 9.40 a. m. Bitten by ten infective *A. ægypti* of lot 104, which had fed eighteen days previously on an experimental case (A.V. 55–S), during the first day of dengue fever; temperature 98°, leukocyte counts normal (7,400). November 20, 8 a. m., bitten by nine *A. ægypti*, lot 104; temperature 98.2°, leukocytes 6,800. November 21, 8 a. m., bitten by nine *A. ægypti*, lot 104; temperature 98°, leukocytes 8,100. November 22, 8 a. m., bitten by ten *A. ægypti*, lot 104; temperature 98.4°, leukocytes 7,350. November 23, 8 a. m., bitten by thirty-five *A. ægypti*, lot 104; temperature 98°, leukocytes 8,100. November 24, 8 a. m., bitten by nine infective *A. ægypti*, lot 100, which had fed twenty-four days previously on an experimental case (A.V. 55–S), during the first day of dengue; temperature 98.4°, leukocytes 6,800. November 25, 8 a. m., bitten by ten *A. ægypti*, lot 100; temperature 97.9°, leukocytes increased to 11,200. November 26, 8 a. m., bitten by ten *A. ægypti*, lot 100; temperature 97.3°, leukocytes 10,650. November 27, 8 a. m., bitten by eight *A. ægypti*, lot 100; temperature 97.7°, leukocytes 9,900. Following this ninth feeding of infective mosquitoes, the volunteer remained well for at least five months. There was no elevation of temperature and no leukopenia during ten days following the last biting. Comment: It is apparent that this man possessed a strong protective immunity against infection with dengue.

EXPERIMENTS TO DETERMINE THE POSSIBILITY OF PRODUCING SYMPTOMLESS DENGUE IN APPARENTLY IMMUNE PHILIPPINE SCOUT SOLDIERS LIVING IN ENDEMIC AREAS

Experiment 7.—March 25, 1929. Four volunteers, F.V. 13, Aguinaldo, Vicente; F.V. 14, Novilla, Filomeno; F.V. 15, Villeza, Cipriano; and F.V. 16, Villarin, Candido, were admitted to the experimental ward at Sternberg Hospital. The following day (March 26) they were each bitten by ten infective *A. ægypti* of lots 9 and 10. As shown by their clinical records, including daily temperature and leukocyte counts, there was no clinical evidence of dengue during the periods of subsequent observation.

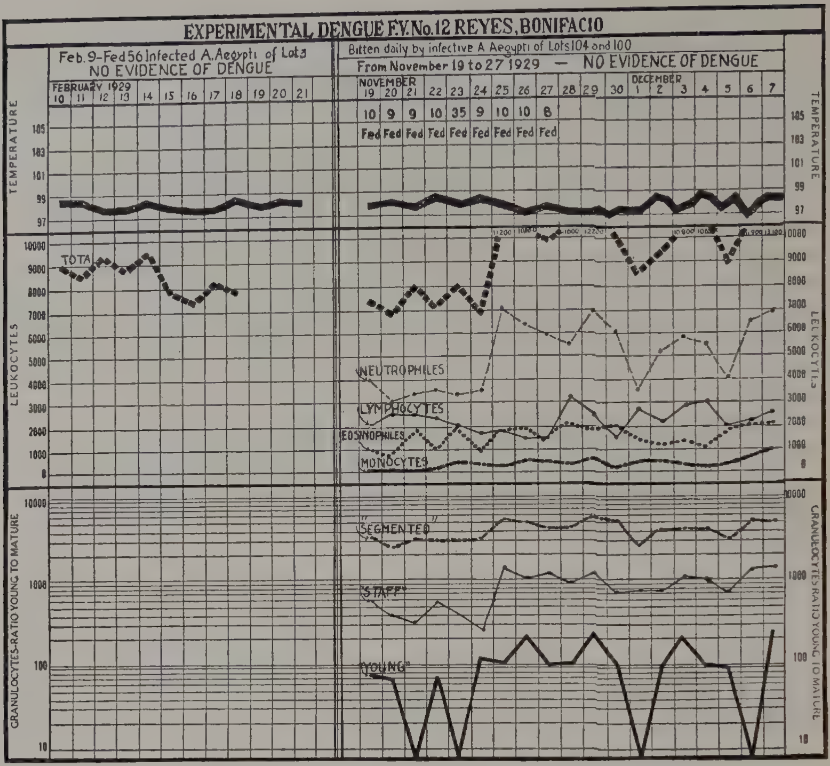

Fig. 41. Experimental dengue in Filipino volunteer 12.

(*a*) *Filipino volunteer 13.*—Aguinaldo, Serial No. 6612777, Ilocano, born in Ilocos Norte, age 29, service in Army six years at Fort William McKinley. He denied ever having had dengue or other diseases. However, an old hospital chart shows that he at one time had a positive Wassermann reaction and had received treatment. At present there is no indication of syphilis. Intestinal parasites were found in the fæces. March 25, 1929, he was bitten by seventy-nine *A. ægypti*, of lot 10; and, as indicated by the subsequent temperature reactions and leukocyte counts, there was no clinical evidence of infection.

(*b*) *Filipino volunteer 14.*—Novilla, Filomeno, Pvt., Co. "E," 57th Infantry, born in Malag, Capiz, age 24, service in Army six months at Fort William McKinley. Had "trankaso" during childhood but states that he has had no other illness. Physical examination and laboratory tests showed him to be normal. March 25, 1929, bitten by one hundred twenty-three dengue-infective *A. ægypti* of lot 10. Both the temperature reaction and leukocyte counts remained normal for ten days.

(*c*) *Filipino volunteer 15.*—Villeza, Cipriano, Pvt., 35th Ambulance Co., 12th Medical Rgt., born in Lubao, Pampanga, age 26, service in Army seven years at Fort William McKinley. Denied having had dengue or other diseases, except operation for stone in bladder or kidney. Physical examination at present time showed no abnormality, except intestinal parasites, glandular enlargement, and a doubtfully positive Wassermann reaction. March 25, 1929, he was bitten by fifty-eight dengue-infective *A. ægypti* of lot 9, after which the temperature and leukocyte counts remained normal for at least ten days.

(*d*) *Filipino volunteer 16.*—Villarin, Candido, Pvt., Co. "E," 57th Infantry, born in Dumarao, Capiz, age 28, service in Army nine years at Fort William McKinley. Previous medical history negative, except for arthritis in 1926. Present examination negative, except for intestinal parasites. March 26, 1929, he was bitten by thirty-seven dengue-infective *A. ægypti* of lot 9, after which both temperature and leukocyte counts remained normal for at least ten days.

DAILY TESTS FOR VIRUS IN THE BLOOD OF FILIPINO VOLUNTEERS 13, 14, AND 16 (EXPERIMENT 7) FOR TEN DAYS AFTER THEY WERE BITTEN BY INFECTIVE AËDES.

Test 1.—One cubic centimeter of blood was collected in citrate every day from each of the three Filipino volunteers. March 29 the blood specimens collected during the first three days were pooled and passed through a preliminary type of Mandler filter, after which 0.5 cubic centimeter was injected subcutaneously into a susceptible American volunteer (A.V. 5–M). Dengue did not develop.

Test 2.—Blood specimens collected from each of the three Filipinos on the fourth, fifth, and sixth days after they were bitten by infective mosquitoes were pooled, filtered, and injected into a susceptible American volunteer (A.V. 6–R) with similar negative results.

Test 3.—Blood collected from each of the Filipinos on the seventh, eighth, ninth, and tenth days was filtered and injected into an American volunteer (A.V. 7–M). He was later shown to be immune.

Test 4.—Normal *A. ægypti* were fed on each of the three Filipino volunteers each day throughout the experiment. April 16 all of the one-, two-, and three-day mosquitoes were combined and called lot 13; and eighty-three fed on a susceptible American volunteer (A.V. 8–G). Dengue did not develop.

Test 5.—April 20, ninety-three *A. ægypti* of the combined groups designated lot 14, which had taken blood from the Filipinos on the fourth, fifth, and sixth days, fed on a susceptible American volunteer (A.V. 9–D) with similar negative results.

Test 6.—April 23, one hundred sixty *A. ægypti* of the combined lots designated as lot 15, which had taken blood from the Filipinos on the seventh, eighth, ninth, and tenth days, bit a susceptible American volunteer (A.V. 12–S) and failed to cause dengue.

Comment.—It is apparent that the immunity of these Filipinos was sufficient not only to prevent the development of symptoms of dengue, but to prevent sufficient development of the virus to infect normal mosquitoes.

Experiment 8.—August 6, 1929. Ten Philippine Scout soldiers were each bitten by from eight to ten infective *A. ægypti* of lots 30 and 32, which had transmitted dengue to American volunteers (A.V. 20–B and A.V. 28–P, respectively). Six days later, August 12, each of these Fili-

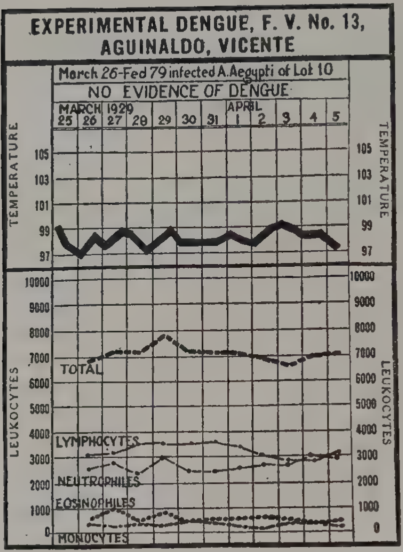

FIG. 42. Experimental dengue in Filipino volunteer 13.

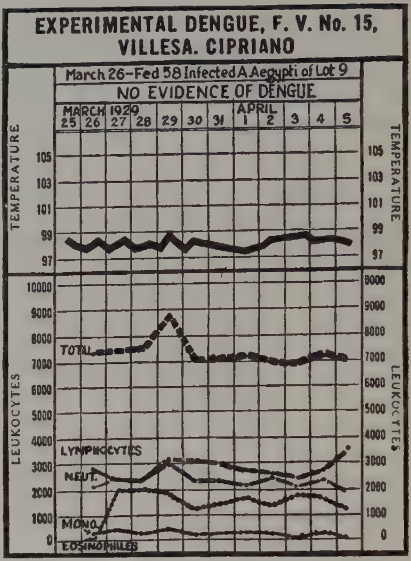

FIG. 44. Experimental dengue in Filipino volunteer 15.

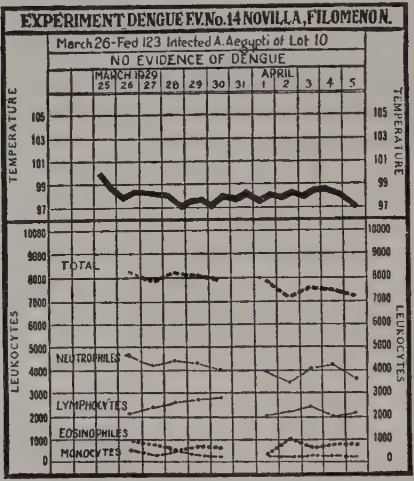

FIG. 43. Experimental dengue in Filipino volunteer 14.

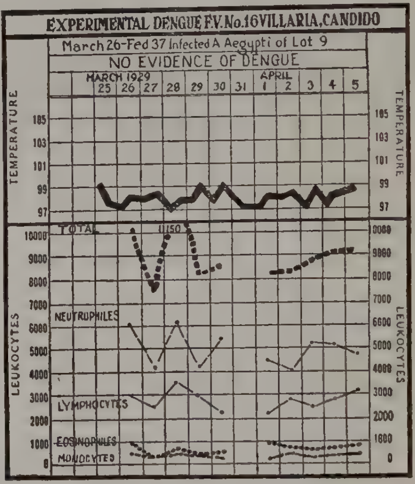

FIG. 45. Experimental dengue in Filipino volunteer 16.

pinos was inoculated intravenously with 0.5 cubic centimeter amounts of citrated infective blood collected about two hours earlier from an experimental case of dengue (A.V. 27–P), during the first day of the disease. Records of daily temperatures and total leukocyte counts were made between August 6 and 21. Several of the men had temperatures as high as 99 to 99.8° F. at irregular intervals, but none of them complained of any illness.

In two instances the leukocyte counts were less than 6,000 on the third and fourth days. There was no clinical evidence to indicate that any of these men developed dengue, either from the bites of infective mosquitoes or from the subsequent intravenous inoculations with infective dengue blood.

Nevertheless, different groups of normal *A. ægypti*, called lots 93 *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, and *i*, respectively, were allowed to feed daily on each man during the third, fourth, fifth, and sixth days after the bitings with infective mosquitoes, while other groups designed as lots 94 *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, and *i*, respectively, were fed on the ten men during the fourth, fifth, sixth, and seventh days after the blood inoculations.

The ten cages containing the various sublots of *A. ægypti* No. 93 were applied August 26 and again September 5 to the arms and legs of a susceptible American volunteer (A.V. 37–M). On the latter date forty-five, forty-three, forty-five, thirty-seven, forty-one, forty-two, thirty-eight, twenty, thirty-six, and fifty of lot 93 mosquitoes, respectively, took blood. As dengue did not result from these feedings, and the volunteer was later proven susceptible, it was considered likely that infective virus had not been present in the blood of the Filipinos, on the third, fourth, fifth, or sixth days after the biting experiments.

Eight of the ten sublots of No. 94 were tested similarly for infectivity September 5 by feeding them on a susceptible volunteer (A.V. 41–F). Of the respective sublots, twenty-eight, thirty-seven, thirty, twenty-six, forty-one, twenty-five, forty-two, and eighteen mosquitoes bit this volunteer but failed to cause dengue. The susceptibility of A.V. 41–F was later proved by the fact that he developed typical dengue following the feeding of a known infected lot of *A. ægypti*.

Comment.—It is believed that these ten Filipinos were completely immune to dengue.

From these various experimental attempts to produce dengue in adult Filipinos living in endemic areas, it may be concluded that such individuals are usually immune and that their immunity is sufficiently strong to protect them against relatively large amounts of infective virus.

(B) THE SUSCEPTIBILITY TO DENGUE OF NATIVES LIVING IN LOCATIONS WHERE THE DISEASE IS NOT ENDEMIC

In order to obtain more information as to whether immunity to dengue might be a natural racial characteristic of natives of the Philippines, transmission experiments were carried out with other natives living near Baguio in the mountains of northern Luzon, a locality that is supposed to be free from dengue. Before these experiments were begun an Igorot was seen suffering with dengue contracted in the lowlands. His history was as follows:

A case of naturally acquired dengue in a Bontoc Igorot.—April 17, 1929, a Bontoc Igorot boy, Howard Fomokao, about 22 years of age, reported to the hospital at Camp John Hay, Mountain Province, with a temperature of 100.2° F., a pronounced generalized punctate and confluent macular rash, and a typical history of dengue. A leukocyte count taken at this time indicated a definite leukopenia; total 4,550, lymphocytes 58 per cent, and granulocytes 42 per cent. Other examinations were negative. This individual had lived in Bontoc at an elevation of over 5,000 feet, until 1919, when he moved to Baguio (elevation about 5,000 feet). He stayed in Baguio until 1927 when he went to Fort William McKinley (elevation not more than 125 feet) where he worked as a "house-boy." He was never ill until Sunday, April 14, 1929, at which time he had started to return to Baguio. During the trip up the mountain he became feverish and dizzy, his eyes hurt, and all his bones and joints ached. There was no rash at that time. Monday, April 15, the aching was less severe, but his eyes burned and the calves of his legs felt stiff. Tuesday, April 16, he had no pain, but felt weak and dizzy. He was seen at Camp John Hay Wednesday (April 17) during the last day of a typical case of dengue. The following day (Thursday, April 18) his temperature was normal and he felt well; however, his leukocyte count was still low: Total cells 3,975, lymphocytes 45 per cent, granulocytes 50 per cent. Blood collected this date was sent by mail to Manila where it was inoculated into a susceptible volunteer with negative results. However, this failure does not indicate that the infection was not dengue, as the blood was collected late in the disease and was not inoculated for at least thirty-six hours after collection.

Comment.—The susceptibility of this Igorot, indicated by a typical case of dengue was, no doubt, due to the fact that he had spent all of his previous life in a dengue-free locality and had not been infected previously. Dengue was contracted at Fort William McKinley or in Manila and developed during his return to the mountains.

EXPERIMENTAL

After some difficulty four of the rather primitive Ifugao boys, from the barrio at Camp John Hay near Baguio, agreed to volunteer for the following transmission experiments.

Experiment 9.—April 21, 1929, 5 p. m. F.V. 1– "Thomas," an Ifugao Igorot boy about 15 years old, who was born at Banaui and had never been out of the mountains, was bitten by seven infective *A. ægypti*, lot 9, which fifty-six days earlier had ingested blood from an experimental case of dengue (A.V. 3–A), during the first day of the disease. Mosquitoes of this same lot had already been proved infective by transmission experiments with an American volunteer (A.V. 6–R). After an incubation period of six days, Thomas developed typical symptoms of a severe case of dengue. The temperature reached a peak of 103.4° F. the afternoon of April 27 and after a gradual decline to normal April 30 again rose to 103.6° May 2. The total leukocyte counts, which had been high during the incubation period, showed a steady decrease after the beginning of the fever, reaching 4,700 the afternoon of April 30. Headache, and pain in the eyes and joints, were extreme. April 28, both parotid glands became so swollen as to suggest the appearance seen in mumps, but this swelling subsided within a few days. The duration of the fever was five and one-half days.

Experiment 10.—April 21, 1929, 5 p. m. F.V. 2– "Banig," an Igorot boy about 17 years old, who was born at Banaui, and had never been out of the mountains, was bitten by six infective *A. ægypti*, lot 9. At the time of this biting Banig had a respiratory infection with a temperature of 101° F. and a leukocyte count of 10,000. By April 25 both temperature and leukocyte counts were normal. After an incubation period of five and one-quarter days dengue began the afternoon of April 27, with headache, pain in eyes, bones, and joints, and a temperature of 101.2° F. From this time on there was a steady decline in the total leukocyte count, which was 4,700 the morning of April 29. In this case the fever lasted four days.

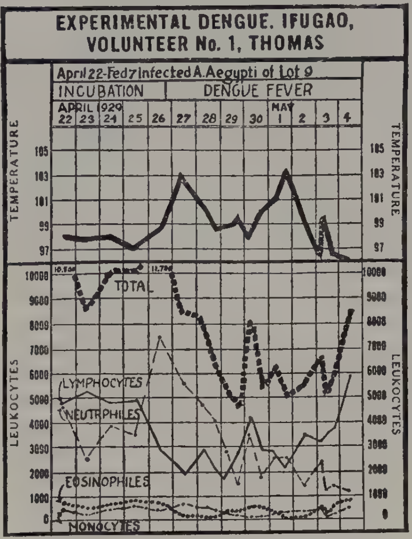

FIG. 46. Experimental dengue in Ifugao volunteer 1.

Experiment 11.—April 21, 1929, 5 p. m. F.V. 3– "Pusayen," an Ifugao Igorot boy about 17 years old, who was born at Banaui and had never left the mountains, was bitten by six infective *A. ægypti*, lot 9. The following day the ring forms of *Plasmodium falciparum* were found in blood smears. After an incubation period of five and one-quarter days dengue began and Pusayen was admitted to the hospital with headache, backache, pains in the eyes, temperature 102.4° F., and a leukocyte count of 5,900. There was some enlargement of the parotid glands. The fever persisted, and April 28 Pusayen had profuse nose bleed twice during the afternoon. The morning of May 1 he was discharged from the hospital but returned that afternoon with renewal of symptoms and was readmitted the next day. The duration of fever in this case was five days. Leukopenia lasted for five days and reached a low point of 4,600.

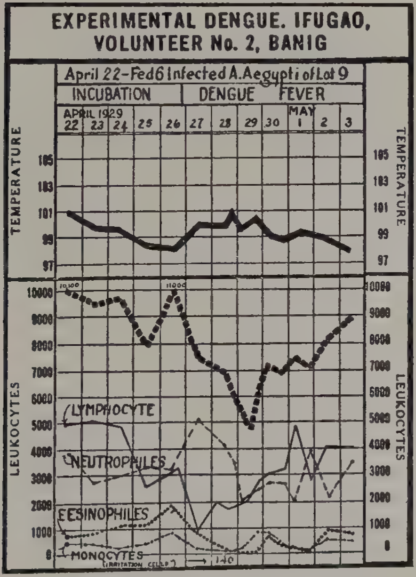

FIG. 47. Experimental dengue in Ifugao volunteer 2.

Experiment 12.—April 26, 1929. F.V. 4– "Basilan," an Ifugao Igo-

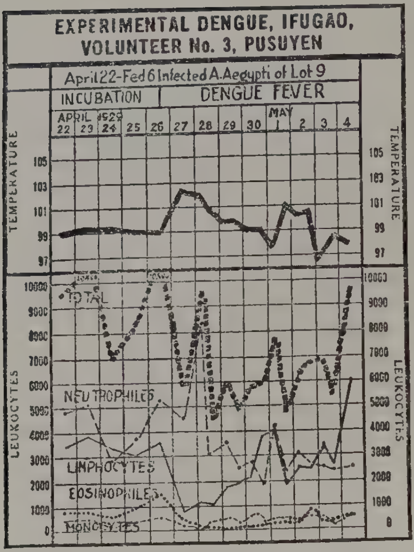

FIG. 48. Experimental dengue in Ifugao volunteer 3.

rot boy about 17 years old, who was born in Banaui and had never been out of the mountains, was bitten by five infective *A. ægypti*, lot 9. After about four days incubation dengue fever developed. The exact time cannot be determined accurately as "Basilan" failed to report April 30 and May 1, and stated that he had been sick in his house in the barrio. When admitted to hospital May 2, he had symptoms of dengue which persisted for four more days.

COMMENT

The last four experiments in which a few infective mosquitoes produced typical dengue in each of four natives from a part of the Philippines that is probably free from the disease, indicate that the immunity observed in natives living in endemic areas is probably acquired by previous infection It is believed that this explanation, advanced by Siler, Hall, and Hitchens, is probably correct and that the natives in the vicinity of Manila and elsewhere probably become infected during childhood or early adult life and consequently develop a strong immunity to dengue. It is of interest to note that lower animals, including mice, guinea pigs, rabbits, and monkeys living in places where they may be exposed to dengue have invariably been just as resistant to experimental infection as were the Philippine Scout volunteers, while certain monkeys from Japan

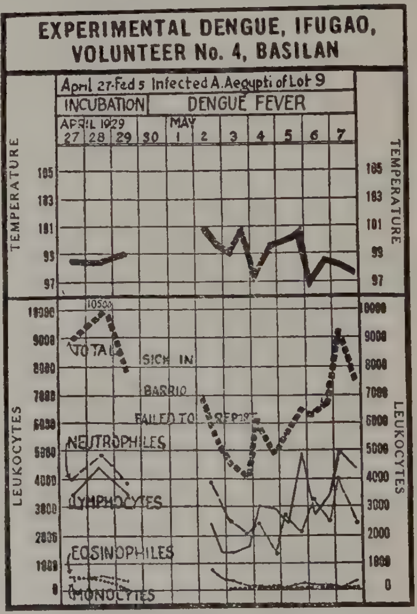

FIG. 49. Experimental dengue in Ifugao volunteer 4.

and from the mountains of northern Luzon have been proved susceptible. In one instance, it was proved that a Japanese monkey (*Macacus fuscatus* 2–W) developed immunity as a result of an infection transmitted by lot 52 *Aëdes ægypti,* about two weeks previously.

Considering the wide distribution of dengue and the many opportunities for mild or unrecognized infections in man and lower animals, it will require more-conclusive evidence than has been offered in the past to prove that immunity to dengue, even in animals other than man and monkey, is natural rather than acquired.

PART II. CLINICAL AND EPIDEMIOLOGICAL OBSERVATIONS CONCERNING THE POSSIBILITY OF REPEATED INFECTIONS WITH DENGUE

For more than one hundred years various observers have called attention to the occurrence of second, third, and fourth attacks of clinical dengue in a certain proportion of individuals. Dengue relapses and recurrences within short periods of time have been reported by Kennedy(15) (1825), Sandwith(1) (1888), Skattowe(8) (1890), Godding(2) (1890), and others. However, Ashburn and Craig(13) in 1907 made the following remarks concerning the possibility of mistakes in reporting recurrences of dengue:

> The correctness of reports of cases in which attacks have occurred a month apart we very much doubt. We had about six patients sent back to us after such periods, supposed to be suffering from second attacks, but in no case was it so. The "second attack" was usually a "malarial proxysm." Cleland(16) (1923) observed, concerning dengue, that one or two relapses may occur after a primary attack, and that second attacks after an interval of a month or longer are probably reinfections; but "neither relapses nor early reinfections are common." He also remarked that "considerable diversity of opinion exists as to the duration of immunity, some considering there is little or none, and others that a previous attack may afford some considerable degree of protection after 21 and even 32 years."

Siler, Hall, and Hitchens(12) (1926), after a careful analysis of the records of several hundred American soldiers stationed in Manila, arrived at the following conclusions:

> Our second epidemiological study indicates that under military conditions in Manila, persons coming from the United States are attacked during their first year to the extent of about 40 per cent. Of those who had one attack, 30 per cent may expect a second; of those who have had two attacks a certain percentage may expect a third; a few may have

four attacks, but our figures are not sufficiently large to make a percentage estimate more than suggestive.

In 1929 the present Research Board made use of a questionnaire sent to a selected group of the leading American and European civilian residents of Manila in an attempt to obtain data concerning their personal experiences with dengue fever. Three hundred seventy-nine individuals who had lived in the Philippines for periods of from one to thirty-five years answered the questions, which included age, birthplace, length and places of residence in the Philippines, number of attacks of dengue in the Philippines and elsewhere, including dates, clinical symptoms, and remarks as to whether or not the diagnosis was made by a physician, and if so whether leukocyte counts were made. The results of an analysis of these reports are shown in Table 22.

The fact that only 63.9 per cent of the "infections" reported were diagnosed by physicians, and that the diagnosis was confirmed by leukocyte counts in only 5.7 per cent, of course, detracts from the value and accuracy of these observations. However, the report furnished by Dr. H. E. Stafford, who has been one of the leading physicians of Manila since the Spanish-American War, is of decided interest. Doctor Stafford stated that he had had two or possibly three attacks of dengue, the first in 1900, the second about 1903, and the third before 1906, each characterized by fever of about five days duration, with aches or pains in the head, eyes, bones, and muscles, moderate aching of the joints, slight chilliness at onset, and no digestive disturbances. Rash did not occur at onset, but was present at end of fever, which was not of the "saddle back" type. The diagnosis was made from the appearance of the rash, and leukocyte counts were not made. He also added the following remark: "This was the usual history of two or three hundred cases under my care in the Civil Hospital when I was chief surgeon of the same."

From the various clinical and epidemiological reports and opinions available on the subject, it appears that there is a widespread belief that many individuals may suffer with more than one attack of dengue in a short period of time. If correct this could indicate that the immunity produced by a single attack may be slight and of short duration.

However, a careful analysis of the clinical records of patients admitted to Sternberg General Hospital between April 1, 1927,

TABLE 22.—*Reinfections with "dengue." Report of personal experiences of residents of Manila.*

Residence in Philippines.	Number of persons.	"Never had dengue."		"Had only one attack."		"Had two attacks."		"Had three attacks."		"Had four attacks."	
Years.			*P.ct.*		*P.ct.*		*P.ct.*		*P.ct.*		*P.ct.*
1	23	10	43.5	13	56.5	-----	-----	-----	-----	-----	-----
2	28	10	35.71	12	42.85	6	21.43	-----	-----	-----	-----
3	31	12	38.70	12	38.70	7	22.57	-----	-----	-----	-----
4	15	5	33.33	6	40	4	26.66	-----	-----	-----	-----
5	18	6	33.3	9	50	3	16.7	-----	-----	-----	-----
6	14	3	21.43	7	50	4	28.57	-----	-----	-----	-----
7	9	2	22.22	1	11.11	3	33.33	3	33.33	-----	-----
8	13	6	46.15	3	23.07	2	15.38	2	15.38	-----	-----
9	9	2	22.22	4	44.44	2	22.22	1	11.11	-----	-----
10	21	6	28.57	5	23.80	7	33.32	3	14.28	-----	-----
11	11	-----	-----	5	45.45	4	36.36	2	18.18	-----	-----
12	2	1	50	-----	-----	-----	-----	1	50	-----	-----
13	3	1	33.3	1	33.3	1	33.3	-----	-----	-----	-----
14	6	2	33.33	1	16.66	1	16.66	2	33.33	-----	-----
15	6	1	16.6	3	49.8	1	16.6	1	16.6	-----	-----
16	8	3	37.5	3	37.5	1	12.5	-----	-----	1	12.5
17	9	4	44.44	3	33.33	1	11.11	1	11.11	-----	-----
18	12	3	24.99	4	33.33	2	16.66	2	16.66	1	8.33
19	11	3	27.27	6	54.54	2	18.18	-----	-----	-----	-----
20	16	6	37.50	4	25	4	25	2	12.50	-----	-----
21	8	1	12.5	4	50	3	37.5	-----	-----	-----	-----
22	7	2	28.57	2	28.57	3	42.85	-----	-----	-----	-----
23	7	3	42.85	4	57.15	-----	-----	-----	-----	-----	-----
24	6	2	33.34	4	66.66	-----	-----	-----	-----	-----	-----
25	6	3	49.98	1	16.66	-----	-----	1	16.66	1	16.66
26	8	3	37.5	5	62.5	-----	-----	-----	-----	-----	-----
27	7	2	28.57	3	42.85	1	14.28	1	14.28	-----	-----
28	8	3	37.5	4	50	-----	-----	1	12.5	-----	-----
29	18	4	22.22	8	44.44	4	22.22	2	11.11	-----	-----
30	16	6	37.50	5	31.25	1	6.25	4	14.1	-----	-----
1–5	115	43	37.3	52	45.4	20	17.3	-----	-----	-----	-----
6–10	66	19	28.78	20	30.3	18	27.27	9	13.63	-----	-----
11–15	28	5	17.85	10	35.71	7	25	6	21.42	-----	-----
16–20	56	19	33.93	20	35.72	10	17.86	5	8.93	2	3.57
21–25	34	11	32.35	15	44.1	6	17.6	1	2.9	1	2.9
26–30	57	18	31.57	25	43.85	6	10.5	8	14.1	-----	-----
31–35	23	10	43.5	6	26.1	3	13.04	2	8.69	2	8.69
1–35	379	125	32.98	148	39.0	70	18.47	31	8.18	5	1.32

and April 1, 1930, fails to entirely substantiate this opinion. The results of this study, which was made by Capt. Mack M. Green, M. C., attending surgeon for the City of Manila, are given below.

AN ANALYSIS OF REINFECTIONS REPORTED AMONG 1258 CASES DIAGNOSED "DENGUE FEVER", AT THE STERNBERG GENERAL HOSPITAL, MANILA, P. I.

"From an analysis of the records of the Sternberg General Hospital, Manila, P. I., between April 1, 1927, and April 1, 1930, it was found that over one thousand two hundred fifty-eight individuals were admitted with a diagnosis of dengue. Eighty-three of these individuals had been readmitted to the hospital at various times and given a second diagnosis of dengue by the ward surgeon, while four came into the hospital a third time with conditions which were called dengue. A careful study of the hospital records of the eighty-three persons readmitted showed that only twenty of them had leukocyte counts below 6,000 during both periods of hospitalization. As a rule only one blood count, taken on the day of admission to the ward, was recorded.

"The following table (No. 23) shows the results of this study.

"As shown above, patients 3 and 20 became ill with symptoms of dengue one week after their discharge from hospital, and it is believed that they may be considered as relapses. Patient 6 had a mild headache, temperature 101, and cramplike pains in abdomen on admission, with no other symptoms. The fever lasted only twenty-four hours and was normal until date of discharge four days later. It is noted from the clinical record that this patient's symptoms began after a midnight meal of sandwiches and beer and it seems quite probable that the diagnosis should have been gastritis, instead of dengue. The diagnosis was presumably made because of the headache and leukopenia. Patient 8 was diagnosed as dengue on three different occasions. The last two admissions, which were separated by an interval of only nine days, probably represented a relapse. Patient 14, on second admission, had a mild headache with no other symptoms. This man remained in the hospital twenty-two days and from a study of the clinical record apparently had a mild back strain. The diagnosis of dengue was made from symptoms of backache and from the white blood count. Patient 15 was a syphilitic case of long standing who had been given four complete courses of treatment with salvarsan and mercury. This patient was admitted several months later to another ward with cerebro-spinal syphilis.

"From a study of these clinical records and from the above findings, I believe it is unwise in a great many cases to make a diagnosis of dengue from a clinical standpoint alone or from a single white blood count.

"Dengue must be differentiated from measles, influenza, cerebro-spinal meningitis, cerebro-spinal syphilis, typhus and typhoid fever, acute rheumatic fever, arthritis, seven-day fever, syphilitic roseola, phlebotomus fever, malaria, and yellow fever.

CONCLUSIONS

1. "This study, based on the hospital records of 1,258 individuals diagnosed as 'dengue' during a period of three years, shows that only 6.5 per cent were subsequently ill with conditions called 'dengue,' and of those readmissions only 1.5 per cent had leukocyte counts below 6,000 during both the first and later illnesses.

2. "A critical review of the records of the twenty patients who had leukopenia during both the first and subsequent admissions to hospital indicate that even in certain of these there was some doubt as to whether dengue had occurred more than once. In two of the individuals the intervals between attacks were so short that they might have represented relapses of the original infection; nine patients had other infections, including coryza, pharyngitis, tonsillitis, pleurisy, gastro-enteritis, and syphilis, which could have caused confusion in the diagnosis, while the remaining nine patients had clinical symptoms of dengue both times. Thus only nine, or 0.072 per cent, of the 1,258 individuals were readmitted for what appeared clinically to be typical reinfections with dengue."

This contribution by Captain Green emphasizes the difficulties often encountered, even under the best of conditions, in arriving at an accurate clinical diagnosis. Because of the added possibility that certain febrile infections showing all of the symptoms, including the rash and the progressive leukopenia considered typical of dengue, may be due to entirely different eitological agents or to denguelike viruses, it is obvious that any final conclusions concerning the specific immunity produced by a single attack of dengue should be based on controlled experimental attempts to reinfect individuals who have previously had typical dengue, caused experimentally by a known strain of dengue virus.

Several workers have attempted to produce experimental dengue in individuals who, according to their histories, had al-

TABLE 23.—*A clinical analysis of twenty cases reported as dengue reinfections. Based on the records of 1,258 individuals with diagnosis of dengue from Sternberg General Hospital, April 1, 1927, to April 1, 1930.*

[Eighty-three, or 6.59 per cent, of the patients were readmitted with the same diagnosis and only twenty of these cases, or 1.58 per cent, had leukocyte counts lower than 6,000.]

Patient No.	First illness.						Interval between first and second days of illness.	Second illness.						Remarks.
	Duration.		Temperature.		Leukocytes; lowest count.	Complications or additional diagnosis.		Duration.		Temperature.		Leukocytes, lowest count.	Complications or additional diagnosis.	
	Before admission to hospital.	After admission to hospital.	Maximum.	Duration.				Before admission to hospital.	After admission to hospital.	Maximum.	Duration.			
	Days.	*Days.*	°*F.*	*Days.*				*Days.*	*Days.*	°*F.*	*Days.*			
1	5	6	100.4	3	4,700	None	91	6	8	100.2	4	2,550	None	Both admissions were clinically dengue.
2	1	8	101.4	5	4,650	do	210	3	7	101.0	1	5,300	Coryza	Had mild coryza and pharyngitis on second admission.
3	1	4	102.0	2	3,500	do	7	4	6	102.0	3	3,050	None	Second admission is believed to be a relapse of first.
4	1	6	100.2	2	4,450	do	370	1	10	105.0	7	4,950	Pleurisy, acute, left base.	Leukocyte count on admission of second attack 4,950, three days later was 8,050.
5	3	7	102.4	4	3.850	do	30	6	8	103.6	3	4,650	None	Immunity of short duration if both admissions were dengue.
6	0	5	101.0	1	5,250	do	227	1	5	101.6	4	3,800	do	Only symptom on first attack was mild headache and pain in abdomen.
7	1	7	100.4	3	4,150	do	343	3	6	103.6	4	5,200	do	Both admissions were clinically dengue.
8	1	6	102.0	2	4,750	do	165	?	8	104.0	3	5,700	do	Discussed in the text.

9	1	8	102.0	4	2,100	----do----------	369	3	4	100.6	2	5,250	Rhinitis, acute----	The diagnosis of influenza cannot be ruled out on second admission.
10	1	10	102.4	6	4,250	----do----------	27	?	5	101.6	2	5,150	None------------	Immunity of short duration if both admissions were dengue.
11	2	12	101.2	8	4,600	Tonsilitis, acute---	366	2	7	102.4	5	4,450	----do----------	Had mild tonsilitis on first admission.
12	1	9	104.0	7	3,600	None------------	334	3	3	101.4	3	2,800	----do----------	Had temperature of 99 °F. when discharged.
13	2	13	103.0	7	3,800	----do----------	77	2	9	102.4	6	4,250	----do----------	Both admissions were clinically dengue.
14	1	4	102.0	2	4,600	----do----------	433	3	22	101.0	16	4,200	Pharyngitis and sacroilliac strain	Diagnosis of dengue on second admission made from leukocyte count and backache, mild.
15	2	7	102.2	5	5,150	----do----------	30	2	11	100.4	8	5,550	Tonsilitis, acute---	Syphilis.
16	0	9	103.8	6	3,400	----do----------	348	1	5	101.2	4	4,100	None------------	
17	?	7	102.4	3	5,200	----do----------	256	0	5	100.2	2	5,200	----do----------	Mild form of dengue on second admission.
18	1	8	103.6	3	2,800	----do----------	207	2	5	101.4	2	5,800	----do----------	Both admissions were clinically dengue.
19	3	4	102.8	3	3,900	Tonsilitis mild-----	76	4	6	102.0	4	4,350	Bronchitis, mild---	
20	1	4	103.6	3	5,500	None------------	6	2	6	103.0	2	4,350	None------------	Second admission is believed to be a relapse of first.

ready suffered with naturally acquired infections. For example, Ashburn and Craig(13) (1907) induced dengue in one individual who said he had had three attacks, the last, two and one-half years previously. Siler, Hall, and Hitchens(12) (1926) inoculated dengue virus into each of eight individuals, who, according to their clinical records, had suffered with spontaneous attacks diagnosed as dengue fifty to one hundred forty-eight days earlier. As a result of the experimental inoculations, four, or 50 per cent, of these men developed dengue.

PART III. EXPERIMENTAL OBSERVATIONS CONCERNING THE IMMUNITY PRODUCED BY A SINGLE ATTACK OF DENGUE

In striking contrast to the general opinion concerning the shortness of dengue immunity, based on clinical and statistical observations, are the results of the following studies in which individuals who had suffered from experimentally induced attacks of dengue, were later tested for immunity by the inoculation of materials known to contain infective dengue virus. Cleland, Bradley, and McDonald(13) (1918), using infective serum and corpuscles from a case of dengue, inoculated 0.5 cubic centimeter subcutaneously into a volunteer, who twenty-four days previously had had a typical attack, produced by a similar injection of infective materials. This man was found to be immune. Later (1919), these authors(3) inoculated about 0.5 cubic centimeter of diluted infective serum in an attempt to reinfect an individual in whom dengue had been produced experimentally by mosquitoes seven and one-half months previously. Negative results were also obtained in this experiment, indicating that the immunity produced by the experimental attack protected against reinfection for at least 229 days.

Siler, Hall, and Hitchens(12) (1926) inoculated 0.5 cubic centimeter amounts of infective dengue virus into each of eleven volunteers, all of whom had been infected experimentally with dengue from thirty-two to sixty-eight days previously. Of this group one man whose initial dengue had occurred fifty-three days earlier developed a mild infection lasting two days; while the other ten, whose experimental infections had taken place thirty-two, forty, forty-six, forty-eight, fifty-three, sixty, sixty, sixty-seven, and sixty-eight days earlier, were all immune. Manoussakis(17) (1928), after failing to reinfect nine experimental volunteers, concluded that persons who had recently recovered from dengue fever were resistant to doses of infective serum after two months. Blanc and Caminopetros(11) (1929) also considered dengue immunity from an experimental standpoint. They reported that ten months after the experimental production of dengue in twenty-five volunteers, nine of these individuals were reinoculated with large doses of dengue virus. None of them showed any signs of infection following the second inoculation, and it was concluded that dengue probably confers a high degree of immunity which is of considerable duration.

PRESENT INVESTIGATION

During the various studies of dengue conducted by this board since December, 1928, a total of more than eighty-three American volunteers have been infected experimentally with a single strain of virus. Although these men continued to live in a locality where dengue is endemic, only one of them developed what might have been a second infection during the subsequent periods of from one to fifteen months. Several of the volunteers returned to hospital with fever and were suspected as reinfections at the time of admission, but subsequent observation and examination proved that they were suffering from other acute infections rather than dengue. The diagnosis in the one doubtful case was not confirmed. As indicated in Table 24, experimental attempts were made to reinfect thirty-five of the volunteers, all of whom had been infected previously with the same strain of virus transmitted by mosquitoes. While the numbers of mosquitoes used in producing the infections differed in the various experiments, ten or less were used in most of the immunity tests. The intervals between the attacks of dengue and the tests for immunity varied from twelve to four hundred days. One of these men was readmitted to hospital two days after the test with fever due to another cause. Among the thirty-five persons rebitten by infective mosquitoes not a recognizable dengue reinfection occurred.

Comment.—The results obtained in these experiments show that for periods of from one-half to thirteen months after an experimentally induced attack of dengue, 100 per cent of the thirty-five volunteers were immune to reinfection. Therefore, it is believed that the immunity produced by a typical attack of dengue is usually strong and of considerable duration.

TABLE 24.—*Immunity tests following experimental dengue fever.*

Experiment No.	Volunteer.	Volunteers tested for immunity.									Results.
		Infected by—				Days elapsing	Retest with—				
		Number fed.	Species of mosquitoes.	Lot No.	Date.		Number fed.	Species of mosquitoes.	Lot No.	Date.	
1	A.V. 3–A	56	*Aëdes ægypti*	3	Feb. 19, 1929	70	8	*Aëdes ægypti*	9–10	May 1, 1929	Immune.
2	A.V. 4–Y	56	do	2	Feb. 19, 1929	86	8	do	12	May 16, 1929	Do.
3	A.V. 24–H	10	*Aëdes albopictus*	29	Aug. 7, 1929	180	8	do	135	Feb. 3, 1930	Do.
4	A.V. 38–H	203	*Aëdes ægypti*	92	Sept. 5, 1929	151	8	do	135	Feb. 3, 1930	Do.
5	A.V. 39–M	47	do	44–47	Sept. 5, 1929	151	10	do	135	Feb. 3, 1930	Do.
6	A.V. 13–W	85	do	18	May 17, 1929	262	10	do	135	Feb. 3, 1930	Do.
7	A.V. 30–L	16	do	16	Aug. 17, 1929	171	19	do	135	Feb. 4, 1930	Do.
8	A.V. 33–B	33	*Aëdes albopictus*	49	Sept. 14, 1929	143	14	do	135	Feb. 4, 1930	Do.
9	A.V. 29–J	(b)	*Aëdes ægypti*	32	Aug. 17, 1929	171	8	do	135	Feb. 4, 1930	Do.
10	A.V. 2–M	15	do	1	Dec. 31, 1928	400	9	do	135	Feb. 4, 1930	Do.
11	A.V. 4–Y	56	do	2	Feb. 19, 1929	351	8	do	137	Feb. 5, 1930	Do.
12	A.V. 3–A	56	do	3	Feb. 19, 1929	351	15	do	137	Feb. 5, 1930	Do.
13	A.V. 36–V	28	do	3	Sept. 21, 1929	137	9	do	137	Feb. 5, 1930	Do.
14	A.V. 32–W	22	*Aëdes albopictus*	43	Aug. 26, 1929	171	14	do	137	Feb. 11, 1930	Do.
15	A.V. 34–S	2	do	43	Aug. 31, 1929	164	9	do	137	Feb. 11, 1930	Do.
16	A.V. 14–A	12	*Aëdes ægypti*	19	June 20, 1929	268	14	do	161	Mar. 15, 1930	Lot not proved infected.
17	A.V. 20–B	9	do	30	July 29, 1929	229	11	do	166	Mar. 15, 1930	Do.
18	A.V. 25–D	3	*Aëdes albopictus*	29	Aug. 6, 1929	220	10	do	167	Mar. 14, 1930	Immune.
19	A.V. 26–T	8	do	29	July 29, 1929	245	15	do	174	Mar. 31, 1930	Do.
20	A.V. 55–S	8	*Aëdes ægypti*	71	Oct. 26, 1929	172	7	do	190	Apr. 16, 1930	Do.
21	A.V. 76–M	61	do	137	Feb. 1, 1930	76	6	do	190	Apr. 16, 1930	Do.
22	A.V. 56–K	25	do	104	Nov. 16, 1929	151	9	do	190	Apr. 16, 1930	Do.
23	A.V. 54–K	54	do	97	Oct. 31, 1929	167	10	do	190	Apr. 16, 1930	Do.

24	A.V. 6–R	49	do	9	Apr. 11, 1929	369	10	do	190	Apr. 16, 1930	Do.
25	A.V. 75–P	60	do	138	Feb. 3, 1930	71	7	do	190	Apr. 16, 1930	Do.
26	A.V. 46–L	10	do	52	Oct. 2, 1929	196	9	do	190	Apr. 16, 1930	Do.
27	A.V. 57–E	30	do	96	Nov. 11, 1929	156	9	do	195	Apr. 17, 1930	Do.
28	A.V. 37–M	42	do	53	Sept. 14, 1929	215	5	do	195	Apr. 17, 1930	Do.
29	A.V. 77–E	87	do	162	Feb. 17, 1930	65	11	do	195	Apr. 23, 1930	Do.
30	A.V. 50–C	14 and 1	do	75 and 80	Oct. 21, 1929	186	15	do	195	Apr. 25, 1930	Do.
31	A.V. 61–J	84	do	116	Dec. 7, 1929	139	7	do	195	Apr. 25, 1930	Do.
32	A.V. 66–W	109	do	114	Dec. 10, 1929	136	5	do	195	Apr. 25, 1930	Do.
33	A.V. 53–C	6 15 4 6 15 9	do	82 83 84 85 88 89	Oct. 28, 1929	179	5	do	195	Apr. 25, 1930	Do.
34	A.V. 48–H	64	do	71	Oct. 21, 1929	189	12	do	195	Apr. 28, 1930	Do.
35	A.V. 41–F	6	do	52	Oct. 7, 1929	203	8	do	195	Apr. 28, 1930	Do.
36	A.V. 47–D	50	do	69	Oct. 7, 1929	12	11	do	52	Oct. 19, 1929	Do.
37	A.V. 72–E	11 56 65	do	125 126 145	Jan. 27, 1930	12	8	*Aëdes albopictus*	128	Feb. 7, 1930	Do.
38	*Macacus fuscatus* 2–W.	44	do	52	Oct. 7, 1929	(a)					

[a] Twenty-six days later, November 1, 5 cubic centimeters of dengue blood from A.V. 51–S were injected.

[b] Filtrates of 50.

DISCUSSION

No evidence has yet been advanced to prove that all people of any race or group may be naturally immune to dengue. This statement probably applies also to many of the published observations concerning the immunity of lower animals. Following the introduction of dengue into a locality, a large proportion of the inhabitants become infected; and in places where dengue has existed for some time a large proportion of the adult natives and the lower animals are found to be resistant to the infection. This is the case in the Philippines where adult natives and lower animals living in endemic areas have been shown to be immune to dengue, while both natives and monkeys from dengue-free areas in the mountains a few hundred miles away are susceptible.

There is no information available to indicate whether the immunity of the adults living in endemic areas is due to a single previous attack of dengue during early life, or whether it has been built up as the result of a series of infections, and maintained by repeated subsequent exposures. It should be interesting to test the immunity of residents of dengue-free areas at intervals, after experimental infection. From clinical and epidemiological studies of susceptible individuals after their arrival in endemic areas, many observers have concluded that an attack of dengue often confers only slight immunity of short duration, and that a certain proportion of such individuals may have second, third, or even fourth attacks within a few years. However, the analysis of clinical records of 1,285 cases, contributed by Captain Green, indicates that second and third clinical attacks during a three-year period may be relatively infrequent.

Certain observers have succeeded in infecting individuals who according to their histories or official records had had previous attacks of dengue. However, from the experimental results reported by Cleland, Bradley, and McDonald,(3) Siler, Hall, and Hitchens,(12) Manoussakis,(17) Blanc and Caminopetros,(11) and the observations of the authors, it is believed that a single attack of dengue in a person living in an endemic locality, confers protective immunity of considerable strength and duration.

SUMMARY AND CONCLUSIONS

1. A large proportion of human beings are apparently naturally susceptible to dengue. This is indicated by the fact that in different epidemics from 75 to 100 per cent of the in-

habitants of various localities have been attacked, particularly in places where the disease is first introduced.

2. American soldiers from various parts of the United States where dengue is not endemic are known to be extremely susceptible to natural dengue infections and to experimental inoculations with the virus, while Americans who have previously lived in endemic localities are usually immune.

3. It has been observed in various parts of the world that most adult natives who have passed through an epidemic of dengue or who live in endemic areas are apparently immune. That this is true of Filipinos living in endemic areas is indicated by the low prevalence of dengue among Philippine Scout soldiers, and by the transmission experiments performed by Siler, Hall, and Hitchens and by the authors, in which between 93 and 100 per cent of over sixty volunteers were immune to inoculations of infective dengue virus.

4. In two instances the immunity of Philippine Scouts was apparently overcome by inoculations with 0.5 cubic centimeter of infective blood intravenously or with 5 cubic centimeters subcutaneously; but such was not the case in other experiments in which large numbers of infective mosquitoes were used.

5. In thirteen experiments it was shown that dengue virus introduced into immune Filipinos not only failed to cause symptoms, but failed to develop in these men, or at most to develop sufficiently to infect normal mosquitoes fed on them during ten subsequent days. This further indicates that the immunity of the natives was well developed.

6. Natives from dengue-free localities in the mountains of northern Luzon were found to be susceptible to natural and experimental dengue infection.

7. Filipinos and monkeys from Manila and other endemic areas of dengue were generally found to be immune, while those living in high altitudes in dengue-free localities (mountains of northern Luzon) were extremely susceptible.

8. Clinical and epidemiological studies have been reported, suggesting that Americans may develop two, three, four, or five attacks of dengue within a short period of time; and that the immunity produced by a single attack may be slight.

9. However, from an analysis of 1,258 hospital records of cases which occurred in Manila between 1927 and 1930, it has been indicated that the proportion of reported recurrences in which the clinical evidence was sufficient to rule out other infections was negligible.

10. Experiments in which thirty-five American volunteers, who had recovered from one attack of dengue produced by a known strain of virus, were reinoculated after periods of one-half to thirteen months gave negative results, indicate that the immunity produced by an attack of dengue probably is stronger and of longer duration than has been generally believed.

11. From these experiments it is, of course, impossible to be sure that the immunity produced by a single attack of dengue may not be maintained by subsequent introduction of virus from infective mosquitoes. This point could be settled by experimental attempts to reinfect individuals living in localities free from dengue.

12. From the results of the present epidemiological, clinical, and experimental studies, it is believed that the immunity following an attack of dengue fever in persons residing in endemic areas, is probably completely protective in a large majority of cases.

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TESTS FOR SPECIFIC ANTIBODIES IN DENGUE

In the following experiments efforts were made to demonstrate specific antibodies in serum obtained from dengue patients and from individuals known to be immune to the disease.

INJECTION OF DENGUE PATIENTS WITH SERUM FROM CONVALESCENTS

Manoussakis(1) (1928) stated that the serum of dengue convalescents failed to confer immunity to susceptible volunteers whether injected before, simultaneously with, or after the infecting dose of dengue virus. Blanc and Caminopetros(2) (1929) also reported that neither the serum nor the whole blood of convalescents had any action on the virus either in vitro or in vivo and that mixtures of such serum and dengue virus were always infective.

Experiment 1.—Two individuals during the first twenty-four hours of typical experimentally produced dengue fever were injected simultaneously with 1 and 2 cubic centimeter amounts of clear serum collected from volunteers one month after their recovery from experimental dengue. The infections continued in apparently unmodified form, and it was concluded that the serum injected had no effect on the course of the disease.

Experiment 2.—In order to determine whether the rash of dengue might be caused to disappear by intradermal injection of serum from convalescents, the following experiment was performed February 21, 1930. Blood was drawn this date, from a recently recovered case of experimental dengue whose temperature had been normal for seven days. The serum was immediately separated and 0.25 cubic centimeter was injected intracutaneously into an area of skin covered with a pronounced secondary, maculopapular rash on a volunteer (A.V. 78–O) during the fourth day of dengue fever. The wheal produced by the injection was pale momentarily, but within a few minutes again became as red as the surrounding skin. There was no fading of the rash during the following twenty hours.

PRECIPITIN TESTS

Experiment 3.—The antigens used in precipitin tests included fresh infective serum collected from experimental cases of dengue during the first day of the disease; and a mosquito filtrate obtained by grinding forty infective *Aëdes ægypti* (lot 25) with sand and 6 cubic centimeters of physiologic salt solution, filtering the suspension through cotton, and clearing the filtrate by centrifugalization. These antigens were used in 0.025 cubic centimeter amounts in small test tubes with different dilutions of serum from immune individuals who had previously been infected experimentally with dengue. No precipitation occurred either in one or eighteen hours.

COMPLEMENT-FIXATION TESTS

Tests were made for complement-fixing bodies in serum from dengue patients and from individuals immune to dengue, using several different antigens prepared with infective blood obtained from dengue patients or from mosquitoes infected with dengue virus.

Experiment 4.—An antigen was prepared with forty infected *A. ægypti* of lot 40 which had fed on an experimental case of dengue (A.V. 20–B) during the first day of the disease. The mosquitoes were macerated and suspended in 8 cubic centimeters of sterile physiologic salt solution, and after the suspension had been filtered through cotton, the filtrate was heated at 56° C. for ten minutes. This antigen, tested in various dilutions, failed to cause any fixation of complement when titrated with serum obtained from individuals who had recently recovered from experimental dengue fever.

Experiment 4–a.—Antigen 2 was prepared with 20 cubic centimeters of infective blood drawn from an experimental case (A.V. 78–O) during the first day of dengue. The blood was laked in distilled water, and centrifugalized at 2,500 revolutions per minute for forty-five minutes. The residue was diluted with a similar amount of mixture composed of equal parts of glycerin and salt solution. When diluted with equal parts of salt solution and used in complement-fixation tests with serum collected from a volunteer on the fourth day of convalescence after dengue fever, this antigen gave anticomplementary reactions. However, when a 1-to-3 dilution was used in 0.5 to 0.3 cubic centimeter amounts with known immune sera the results were negative.

Experiment 4–b.—Antigen 3 was prepared with infective blood drawn from the patient mentioned above (A.V. 78–O). Ten cubic centimeters of the coagulated blood was placed in a mortar, ground with 12.5 cubic centimeters of 95 per cent alcohol and subsequently allowed to stand for seven days. The alcoholic extract, diluted 1 to 5 with salt solution, was used in tests with serum drawn from volunteers (A.V. 24–H, A.V. 25–D, A.V. 27–P, and A.V. 28–P), and from each of the three authors, at relatively short periods after they had recovered from attacks of dengue fever. The results were negative.

Experiment 4–c.—Antigen 4 consisted of recently collected infective serum from an experimental case of dengue. This was tested with serum from individuals who had recently recovered from dengue, with negative results.

Experiment 4–d.—Antigen 5 was prepared with infective *Aëdes ægypti* of lots 9, 12, 17, 18, and 19. Two hundred seventy-nine of these mosquitoes were macerated and suspended in 5 cubic centimeters of 95 per cent alcohol. Twelve days later the alcoholic mosquito extract was cleared by centrifugalization at low speed, after which it was diluted 1 to 10 with physiologic salt solution and used in tests with sera obtained from four volunteers. Three of these individuals had recently recovered from dengue as follows: A.V. 13–W, nine days, and A.V. 3–A and A.V. 4–Y about seventy-nine days previously; while the other volunteer (A.V. 9–D) had been suffering with dengue fever for only one day. Using 0.1 cubic centimeter amounts of the 1 to 10 dilution of antigen and 0.1 cubic centimeter amounts of the four sera the tests resulted as follows: A.V. 4–Y, serum was negative; A.V. 13–W, two plus; A.V. 9–D, three plus; and A.V. 3–A, four plus.

As the remainder of this antigen was lost, another one was prepared similarly with 584 infective *A. ægypti* from various experimental lots. However, all tests with this second mosquito antigen were negative.

Experiment 4–e.—Antigens 6 and 7 were prepared from a mixture of liver and spleen taken from Philippine monkeys (*Macacus philippinensis* 33–D and 34–E) six days after the animals had been bitten by infective *A. ægypti.* The liver-and-spleen mixtures were ground up and suspended in 9 per cent salt solution in the proportion of 1 gram to 0.5 cubic centimeter. After standing overnight in the ice box the suspensions were diluted with nine times their volume of distilled water. These antigens were used in tests with sera from volunteers who had recently recovered from dengue fever. The tests were negative.

CONCLUSIONS

With the possible exception of complement-fixing bodies as noted in experiment 4–d, specific antibodies were not demonstrated in serum collected from various individuals, during and following recovery from dengue fever.

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OBSERVATIONS CONCERNING PROPHYLACTIC VACCINATION AGAINST DENGUE

From investigations in which 100 per cent of thirty-five individuals, experimentally infected with dengue, were later found to be resistant to second inoculations with infective virus, it appears that a single attack of dengue usually produces a strong immunity which may last for thirty to four hundred days and probably longer. This observation suggests the possibility of producing an effective prophylactic vaccine which might be used for the protection of susceptible individuals entering endemic localities, particularly for troops being sent for short tours of service in the Philippine Islands or other tropical countries.

Observations have been made concerning the immunizing action of subcutaneous inoculations with various materials, including noninfectious filtrates of suspensions of infective mosquitoes, dried blood collected from dengue patients during the infective period, and a vaccine prepared from a saline suspension of dengue-infected *Aëdes ægypti.*

EXPERIMENTAL

SUBCUTANEOUS INJECTIONS OF NONINFECTIOUS FILTRATES OBTAINED FROM SUSPENSIONS OF MOSQUITOES INFECTED WITH DENGUE VIRUS

During a series of experiments dealing with the filterability of dengue virus as it exists in infected mosquitoes, the virus failed to pass through diatomaceous filters in twelve instances, as was indicated by the fact that inoculation of the filtrates into susceptible volunteers did not cause dengue. When tested for immunity with infective mosquitoes, these same volunteers, with one exception, developed dengue. However, as the immunity test was applied in the excepted case several months after discharge from hospital, his record is not considered.

SUBCUTANEOUS INJECTIONS OF DESICCATED BLOOD

Several attempts were made to preserve the virus of dengue in frozen, dried blood. The virus was collected from experimental cases during the infectious stage of the disease. The dried blood, sealed in a vacuum in pyrex glass tubes, was stored at 18° C. and, after different intervals of time, five of the specimens were suspended in salt solution and tested for infectivity by subcutaneous inoculation into susceptible volunteers. As none developed dengue from this procedure, it may be assumed that the virus had either died or become attenuated. However, it seemed desirable to ascertain if such inoculations had produced any immunity. When the volunteers were tested with infective mosquitoes eight to eighteen days later, all developed dengue.

VACCINE PREPARED WITH MOSQUITOES INFECTED WITH DENGUE VIRUS

A "mosquito" vaccine (No. 1) was prepared according to the following technic and was tested on susceptible human volunteers. December 20, 1929, two thousand ten infective *Aëdes ægypti* from combined lots 95, 101, 103, 104, 110, 111, 112, 114, 116, 117, 118, 129, 130, 131, and 134, were killed and ground in a sterile porcelain mortar with fine quartz sand and 10 cubic centimeters of salt solution containing 1.6 per cent chemically pure phenol and 0.4 per cent chemically pure formalin. The suspension was transferred to a sterile bottle, and after it had stood for forty-eight hours to allow insoluble particles to settle, 10 cubic centimeters of sterile physiologic salt solution was added to the clear supernatant fluid to further dilute the preservatives. The suspension was then kept at room temperature for eight days to destroy bacteria, after which, 20 cubic centimeters of sterile salt solution was added. After slow centrifugalization, to eliminate the remaining insoluble matter, 37 cubic centimeters of a clear amber-colored fluid was obtained. This fluid, which contained phenol and formalin in final dilutions of 0.4 and 0.1 per cent, respectively, mixed with the extract from about fifty mosquitoes per cubic centimeter, was placed in ampules in 1 cubic centimeter amounts and used as a vaccine. Before use, the vaccine was examined for living bacteria and for bacterial toxins. Anaërobic and aërobic cultures were made and incubated for periods of ten or more days. Although no bacteria were found, portions of the fluid media were inoculated into guinea pigs. The vaccine was also inoculated in 1 cubic centimeter amounts directly into rabbits, guinea pigs, and white mice, all of which remained normal. As a final preliminary test, doses of the vaccine were inoculated subcutaneously into each of the authors before it was used on volunteers.

TABLE 25.—*Effect on dengue immunity of subcutaneous injections of filtrates of suspensions prepared with infected Aëdes ægypti.*

No.	Volunteer.	Inoculations with mosquito filtrates.	Test for immunity with infective mosquitoes.				Date of onset of disease.	Incubation period.	Fever.		Leukopenia.	
			Date.	Result.	Mosquitoes.				Duration.	Height.	Duration.	Lowest count.
					Number.	Species and lot No.						
								Days.	*Days.*	°*F.*	*Days.*	
1	A.V. 20-B	June 29, 1929 July 11, 1929 July 20, 1929	July 29, 1929	Dengue	9	*Aëdes ægypti*, lot 30	Aug. 4, 1929	6.00	3.5	100.4	3.5	4,200
2	A.V. 23-B	July 1, 1929 July 11, 1929 July 20, 1929	July 29, 1929	do	12	*Aëdes albopictus*, lot 29	Aug. 5, 1929	7.00	4.5	101.4	6.0	2,150
3	A.V. 24-H	July 11, 1929 July 21, 1929 (dried dengue blood).	Aug. 7, 1929	do	10	do	Aug. 12, 1929	5.50	3.5	102.0	4.0	5,100
4	A.V. 25-D	July 11, 1929 July 21, 1929 (5-day virus).	Aug. 6, 1929	do	3	do	Aug. 12, 1929	6.25	5.5	103.0	7.0	3,200
5	A.V. 27-P	July 21, 1929 (5-day virus). July 29, 1929	Aug. 6, 1929	do	1	do	Aug. 11, 1929	5.00	6.0	103.2	6.5	2,100
6	A.V. 30-L	Aug. 17, 1929	Aug. 26, 1929	do	16	*Aëdes ægypti*, lot 41	Aug. 30, 1929	4.50	4.0	103.0	7.0	3,250
7	A.V. 64-C	Nov. 29, 1929	Dec. 10, 1929	do	58	*Aëdes ægypti*, lot 117	Dec. 16, 1929	6.00	4.5	102.2	6.0	2,600

TABLE 26.—*Effect of dengue immunity of subcutaneous injections of frozen dried infective blood kept for different periods of time.*

No.	Volunteer.	Inoculation with dried dengue blood.					Volunteer tested for immunity with infective mosquitoes.		
		Date.	Amount of whole blood.	Suspended in—	Filtered.	Age of blood.	Date.	Number.	Species and lot No.
			cc.						
1	A.V. 24-H	July 21, 1929	0.5	Salt solution	Yes	4	Aug. 7, 1929	10	*Aëdes albopictus*, lot 29.
2	A.V. 26-T	July 21, 1929	0.5	do	No	4	July 29, 1929	8	Do.
3	A.V. 28-P	July 11, 1929	1.5	do	Yes	15	July 29, 1929	8	*Aëdes ægypti*, lot 32.
4	A.V. 32-W	Aug. 17, 1929	1.0	do	No	52	Aug. 26, 1929	22	*Aëdes albopictus*, lot 43.
5	A.V. 65-F	Nov. 29, 1929	0.7	do	No	79	Dec. 10, 1929	10	*Aëdes ægypti*, lot 112.

No.	Volunteer.	Volunteer tested for immunity with infective mosquitoes.			Fever.		Leukopenia.		Rash.
		Result.	Date of onset of the disease.	Incubation period.	Duration.	Height.	Duration.	Lowest count.	
				Days.	*Days.*	°*F.*	*Days.*		
1	A.V. 24-H	Dengue	Aug. 12, 1929	5.5	3.5	102.0	4	5,100	Terminal.
2	A.V. 26-T	do	Aug. 4, 1929	6.5	1.5	101.0	4	3,900	Initial.
3	A.V. 28-P	do	Aug. 4, 1929	6.0	4.5	102.6	5	3,700	None.
4	A.V. 32-W	do	Aug. 31, 1929	5.0	5.0	104.6	7	2,150	Do.
5	A.V. 65-F	do	Dec. 17, 1929	6.0	5.5	102.4	6	3,150	Initial and terminal.

Experiment 1.—February 13, 1930. One cubic centimeter of dengue vaccine 1 was injected subcutaneously into the right arm of a susceptible volunteer (A.V. 84–C). The only reaction noted was temporary itching at the point of inoculation. February 17, 1930, another dose of 1 cubic centimeter of vaccine was injected subcutaneously. February 24, 1930, the volunteer was tested for immunity, by allowing twenty-one infective *A. ægypti* of lot 137 to take blood at 10.30 a. m. After an incubation period of five days dengue developed March 1, 1930. The duration of the fever was five days, and the highest temperature was 102.4°, while the leukopenia lasted six days and the lowest count was 3,600. Comment: Two subcutaneous injections of vaccine 1, given eleven and seven days before the feeding of infective mosquitoes, failed to prevent the development of dengue in this volunteer.

Experiment 2.—February 24, 1930, 10.30 a. m. One cubic centimeter of dengue vaccine 1 was inoculated subcutaneously into the arm of a susceptible volunteer (A.V. 83–R). Intense itching was felt about the point of injection for a few minutes, but no other reaction was noted. March 1, 1930, a second subcutaneous injection of 1 cubic centimeter of dengue vaccine 1 was given. March 8, 1930, or twelve days after the initial injection of vaccine, the volunteer was tested for immunity by feeding eleven infective *A. ægypti* of lot 168 at 10 a. m. After an icubation period of five days, dengue fever began March 13. The fever lasted four and one-half days and the highest point reached was 102.6°, while the leukopenia lasted for five days and the lowest count was 2,600. Comment: Two injections of vaccine 1, given at intervals of five days, failed to protect the volunteer when tested one week following the second injection.

DISCUSSION

The negative results obtained in these preliminary experimental attempts to vaccinate susceptible volunteers with noninfectious filtrates obtained from suspensions of recently killed infective mosquitoes, with suspensions of dried infective blood kept for different periods of time, and with vaccine 1 prepared from infective mosquitoes, do not indicate that an effective prophylactic vaccine may not be developed. Because of the invisibility of the virus of dengue, it is impossible to estimate its concentration in the various materials used, or to determine whether it is killed or attenuated. The noninfective Mandler and Berkefeld filtrates, obtained from saline and broth suspensions of macerated infective mosquitoes, probably did not contain infective virus and, as would be expected, injections of this material failed to protect any of the individuals against subsequent infection. The desiccated blood, collected from experimental cases of dengue and frozen immediately, was kept for periods of four to seventy-nine days before use. The fact that this material failed to cause dengue indicates that the virus was either dead or attenuated. While these injections failed to prevent subsequent infection, still some of the volunteers had rather mild cases of dengue. In the vaccine (No. 1) prepared from a saline suspension of infectious mosquitoes, the virus was probably killed, although it may have been attenuated. Used in doses of 1 cubic centimeter, this vaccine failed to immunize volunteers when tested within two weeks after the injections had been given.

The recently reported work of Blanc and Caminopetros(1) (1929) concerning dengue vaccination is of interest. These investigators state that injections of dengue virus, killed by heat or by chemicals, had no protective value against infection with dengue. Therefore, they attempted to obtain an attenuated living virus that might give rise to a low-grade "symptomless" dengue, with subsequent immunity. It was found that dengue virus in blood could be attenuated by mixing it for five minutes with one-twentieth of its volume of bile, while the virus was killed by similar exposure to one-fifteenth of its volume of bile. As it was found that volunteers inoculated with the bile-virus mixture (1 to 15) were no longer susceptible to injections with bile-virus mixtures (1 to 20), these materials were combined and used as a vaccine. Twenty-two persons were doubly inoculated, first with the bile virus (1 to 15) and later with the bile virus (1 to 20); and when tested for immunity three weeks later only two of the individuals developed dengue.

The desirability of an attenuated virus for use as a prophylactic vaccine has also been recognized by the authors, who have considered the possibility of obtaining a constant supply by passage of the virus in series through mosquitoes. However, although there appeared to be some decrease in virulence of the virus after the second passage from mosquito to mosquito, this observation has not yet been confirmed; and consequently there has been no opportunity to attempt to immunize susceptible persons in this manner.

The possibility of developing an effective prophylactic vaccine is considered sufficiently promising to warrant further work on the subject. The development of such a vaccine, suitable for practical use in the immunization of troops before arrival in the Philippines, should make it possible to greatly reduce the incidence of this disease, which is one of the most important causes for loss of time among the military personnel of the Islands.

SUMMARY AND CONCLUSIONS

1. Subcutaneous inoculations of volunteers with filtrates obtained from saline suspensions of macerated infective mosquitoes failed to protect them against subsequent infection with dengue virus.

2. Subcutaneous inoculation of volunteers with saline suspensions of noninfective dried blood, previously collected from experimental cases, failed to protect against subsequent infection with dengue virus.

3. Inoculations with a vaccine prepared from infective *A. ægypti* also failed to produce protective immunity in man within periods of twelve days.

4. It is believed that an effective prophylactic vaccine against dengue may be developed by further investigation.

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DENGUE IN LOWER ANIMALS

I. PHYSICAL SIGNS, TEMPERATURE, AND LEUKOCYTE REACTIONS IN ANIMALS INOCULATED WITH DENGUE VIRUS

The need for a diagnostic laboratory animal suitable for use in experimental studies of dengue led to the following investigation, in which attempts were made to produce dengue in chickens, lizards, guinea pigs, rabbits, goats, and monkeys.

While certain early observers, including Sandwith(1) and Hirsh,(2) reported epizoötics in birds, mice, rats, cats, dogs, and cattle that occurred coincident with outbreaks of dengue in man, investigators who have since attempted to infect lower animals experimentally have usually reported negative or inconclusive results. In 1914 Lavinder and Francis(3) announced experiments in which they inoculated nine *Macacus rhesus* monkeys with blood from dengue patients. In two tests the blood was collected on the second and third days of dengue, but in the other seven tests it was drawn on the third, fourth, or fifth day of the disease, at which time the virus is usually not transferable. Their conclusions were as follows:

> If dengue is conveyable to the rhesus monkey by intravenous or subcutaneous inoculation of defibrinated blood, these animals do not show the disease by changes in temperature, appearance of skin eruption or any of the ordinary symptoms of illness; further, that it is doubtful whether they show any definite and characteristic change in the white cell count, but the results obtained by us are perhaps sufficiently suggestive to invite further effort.

Kraus(4) in 1916 injected blood obtained from dengue patients into guinea pigs which were observed for subsequent periods of eight days. Neither fever nor other symptoms of infection appeared during this time. In 1917 the Japanese investigators Koizumi, Yamaguchi, and Tonomura(5) reported the results of animal experiments conducted in Formosa. They were unable to produce recognizable infections by the inoculation of small amounts of defibrinated blood from dengue patients into white mice, rabbits, dogs, and long-tailed Formosan monkeys. However, guinea pigs inoculated similarly became ill and usually died within seven to thirty-six days. By subinoculation from animals injected fifteen days previously similar symptoms were produced in other guinea pigs, which died within five to nineteen days if inoculated subcutaneously or intraperitoneally, and within twenty-eight to thirty-four days if the inoculation was made intravenously. They were unable to pass the infection through a third series of animals. It is unfortunate that the specific nature of the infection in these guinea pigs was not established.

Cleland, Bradley, and McDonald(6) in 1919 also reported experimental attempts to produce dengue in guinea pigs and rabbits during which they were unable to observe any signs of infection. In one experiment a guinea pig, injected with blood which produced dengue in a volunteer, was tapped at the end of seven and one-half days and 2 cubic centimeters of its blood was injected into a volunteer with negative results. Chandler and Rice(7) in 1923 attempted to infect guinea pigs, white mice, and a young rhesus monkey with dengue, but the results were considered negative. They also made the following observations:

> Furthermore, 65 guinea pigs of all ages were housed in an outdoor pen during the entire summer and fall where yellow fever mosquitoes swarmed. Only one became ill and three days later died from a respiratory infection. These pigs were freely bitten, even tormented by the mosquitoes and the percentage of dengue in that district was high.

They concluded "that guinea pigs are not susceptible to dengue organisms." The rhesus monkey, which they inoculated with about 5 cubic centimeters of citrated dengue blood drawn on the third day of the disease, appeared "chilly and morose" on the third and fourth days after inoculation, but there was no significant rise in temperature. On the fifth day he showed a fine papular rash on the chin and throat which was not considered to be a manifestation of dengue. These workers stated, "we are inclined to think that the inoculation had negative results as did those of Lavinder and Francis (1914); at any rate it was very doubtfully positive." Holt(8) in 1923 failed to produce recognizable evidence of infection in guinea pigs and rabbits inoculated with blood from dengue patients. Armstrong(9) (1923) attempted to convey dengue to guinea pigs, rabbits, white rats, and rhesus monkeys, but with negative results. He stated—

> Animals were injected with blood taken at various stages of the disease (5th hour to convalescence). The animals were observed for from 8 to 30 days in various cases. In no instance was the behavior of the injected

animals different from that of the controls. Blood counts were made in monkeys, rabbits and rats but no variations deemed significant were noted. In the case of the guinea pigs, however, the results were complicated by an epidemic of broncho-pneumonia which broke out among them after about the 15th day. Controls and injected animals were alike attacked.

Harris and Duval(10) in 1924 reported extensive investigations with guinea pigs and claimed to have transmitted dengue through a long series of these animals. However, many of the experimental animals were infected by organisms of the paratyphoid group, and they apparently failed to prove that the reactions described were due to the virus of dengue. Siler, Hall, and Hitchens(11) in 1926, in Manila, injected each of two young hogs intravenously with 10 cubic centimeters of blood from two early cases of dengue. One week later the animals were bled, and the mixed blood injected in 10 cubic centimeter amounts into two other hogs. After one week the latter animals were bled and their blood was inoculated into two susceptible volunteers, who failed to develop dengue from this injection. Counts of leukocytes in the blood of the hogs showed no significant changes.

Manoussakis(12) (1929) reported that guinea pigs inoculated with three times the dose of dengue virus required to infect volunteers showed no recognizable reaction. Blanc, Caminopetros, and Manoussakis(13) (1929) inoculated monkeys, dogs, pigeons, fowls, white rats, rabbits, and guinea pigs but failed to note any febrile or other reactions. However, in two cases serum was taken from guinea pigs about the fourth day after inoculation and injected into human volunteers, both of whom developed dengue after an incubation period of four days. They were not able to transfer the infection through guinea pigs in series. Blanc, Caminopetros, Dumas, and Saenz(14) in 1929 inoculated blood containing dengue virus into several species of Asiatic and African monkeys—macaques, baboons, cercopithecs, and margabeys. None of these animals showed any temperature reaction or clinical signs of dengue. However, when the blood from a certain number of these monkeys was transferred to man, dengue fever developed after an incubation period of four to five days. Hence, the monkeys concerned must have suffered from a latent or nonapparent infection. They drew the following conclusions:

> 1. The macaque monkeys *Cynomolgus fascicularis* and *Cercopithecus callitrichus*, inoculated with blood from human dengue patients suffer from a latent infection. Their blood, which is non-virulent twenty-four hours after inoculation becomes virulent from the 5th to the 8th day. It is no

longer virulent at the 12th day. A single experiment with a baboon yielded a negative result. 2. Monkeys which have suffered with latent dengue develop an immunity lasting at least 50 days. 3. The blood of monkeys infected with dengue is virulent for man. Under experimental conditions it does not seem to be virulent for the monkey, in which neither infection nor immunity develops.

PRESENT INVESTIGATION

In the present study attempts were made to infect chickens, lizards, mice, rabbits, goats, and monkeys with dengue. It was considered advisable to use animals that had been obtained in localities free from dengue or had been protected from mosquitoes since birth, in order to avoid the possibility of selecting animals that had already become immune, because of previous naturally acquired infection. However, such "protected" animals were not always available, and many of the experiments were done with animals obtained from the Bureau of Science in Manila.

Many of the preliminary experiments were limited to observations on the temperature and leukocyte reactions before and after inoculations with materials known to contain infective dengue virus. In later experiments attempts were made to demonstrate infection in the inoculated animals regardless of symptoms, by feeding normal *Aëdes* mosquitoes on them and later testing the mosquitoes for infectivity by allowing them to feed on volunteers.

EXPERIMENTAL

1. CHICKENS

The chickens used experimentally were obtained in the city market in Manila and kept in the laboratory for about one week.

Experiment 1.—February, 1929. Two cockerels and four chicks were inoculated subcutaneously with blood containing infective dengue virus and with saline suspensions of infected mosquitoes. The daily temperature and leukocyte counts on the cockerels did not indicate infection; and all of the chickens remained normal in appearance.

Experiment 2.—August 31, 1929. Four cockerels, Nos. 3, 4, 5, and 6, were inoculated into the "wattle" with 0.5 cubic centimeter each of infective blood collected from an experimental case of dengue (A.V. 31–D) during the first day of fever. The daily temperature reactions and leukocyte counts failed to indicate any infection.

Groups of normal *A. ægypti* fed on the chickens three, four, and five days after the inoculations were combined and designated lot 55. Similar groups fed on the sixth, seventh, and eighth days were combined and called lot 56. These two lots, of mosquitoes Nos. 55 and 56, were tested September

26, when one hundred twenty and one hundred five, respectively, fed on a susceptible volunteer (A.V. 41–F) but failed to cause dengue.

Comment.—Chickens obtained from Manila, where dengue is endemic, failed to show any recognizable reactions, following inoculations with dengue virus. Normal *A. ægypti* fed daily on such chickens between the third and eighth days after inoculation failed to transmit dengue to human volunteers.

2. LIZARDS

Two young lizards, caught in a dwelling in Manila, were each inoculated subcutaneously August 31, 1929, with 0.5 cubic centimeter amounts of blood collected from an experimental dengue case (A.V. 31–D) during the first day of the disease. So far as could be observed by their appearance the lizards remained well. They were liberated about one month later.

3. WHITE MICE

Experiment 1.—December 17, 1928. Two white mice raised in an unscreened cage in the City of Manila, were each inoculated subcutaneously with 0.5 cubic centimeter amounts of infective blood from a case of dengue (Keller). These animals showed no physical signs of illness.

Experiment 2.—January 9, 1929. Two white mice were each inoculated intraperitoneally with 0.5 cubic centimeter amounts of infective blood from A.V. 2–M. Two other mice were injected similarly with blood from A.V. 1–H. These mice failed to show any physical signs of illness.

Comment.—Subcutaneous and intraperitoneal inoculations of infective blood from experimental cases of dengue into white mice raised in an unscreened cage in the City of Manila, failed to cause any recognizable illness.

4. RABBITS

The rabbits used were obtained from an unscreened animal house in the Bureau of Science, Manila, and had, therefore, probably been exposed to the bites of dengue-infected mosquitoes for indefinite periods of time.

Experiment 1.—December 17, 1928. Two rabbits each received subconjunctival inoculations, into the left eye, of 0.1 cubic centimeters amounts of infective serum from a proved case of dengue (Keller). As a control the left eye of each rabbit was inoculated similarly with serum from a patient suffering with pneumococcus septicæmia. Neither of the animals showed any significant reaction during two weeks observation.

Experiment 2.—February 7, 1929. A rabbit (No. 1) was inoculated intracranially with 0.25 cubic centimeter of a suspension made by macerating two infective *A. ægypti*, lot 1, and two of lot 2, in 1.5 cubic centimeters of physiologic salt solution. After inoculation the rabbit showed a slight decrease in rectal temperature until the fourth day, after which it rose and remained at about the height noted on the day of inoculation until the tenth day when it reached 104° F. The leukocyte curve dropped gradually from 8,500 on the day of inoculation to 5,600 on the ninth day; and there was an abrupt drop in the neutrophilic granulocytes on the fifth, sixth, and seventh days. On the seventh day the hind quarters of the rabbit became paralyzed. The animal was killed and examined February 19. There was no evidence of infection and the paralysis was attributed to traumatism due to the method of inoculation.

TABLE 27.—*Rabbit 1 (gray rabbit).*

	Days after subdural inoculation with dengue-infected mosquitoes.												
	0	1	2	3	4	5	6	7	8	9	10	11	12
Temperature °F	103.0	102.4	101.6	101.8	101.6	103.2	103.4	103.0	103.2	103.6	104.0	104.0	104.6
Total leukocytes	8,500	9,600	8,200	9,100	8,000	7,900	7,600	7,800	6,500	5,600	7,500	13,300	9,400
Neutrophiles	3,800	5,800	3,900	3,700	3,000	1,300	1,200	1,700	2,300	2,300	2,500	5,700	3,100
Lymphocytes	5,000	3,600	4,100	4,600	4,600	6,100	5,000	5,300	3,700	3,000	4,700	6,500	5,000
Monocytes	250	200	250	270	480.0	320	1,400	630	460	50	320	900	1,300

As a control on this experiment, another normal rabbit (No. 1–A) was inoculated similarly with a suspension of normal mosquitoes. This animal also showed irregular daily temperature and leukocyte reactions, as indicated in Table 28.

TABLE 28.—*Rabbit 1–A.*

	Days after subdural inoculation with normal mosquitoes.												
	0	1	2	3	4	5	6	7	8	9	10	11	12
Temperature °F	103.8	101.2	101.0	101.4	102.1	102.6	104.0	101.2	101.0	104.0	102.8	103.7	102.5
Total leukocytes	7,900	10,000	7,900	8,500	7,700	7,800	13,300	7,500	8,900	8,500	7,500	10,700	8,300
Neutrophiles	4,200	6,500	2,300	3,200	1,600	2,400	2,500	1,300	1,200	680	1,400	1,300	1,450
Lymphocytes	3,700	3,300	5,300	4,700	4,700	5,000	7,000	5,700	6,200	6,600	4,100	7,850	5,600
Monocytes	150	300	240	670	1,400	460	3,200	530	1,300	1,200	910	1,500	1,400

Experiment 3.—February 19, 1929. An adult rabbit (No. 2), which since February 9 had received daily intravenous inoculations with 3 cubic centimeters of a 3 per cent suspension of Higgins' India ink in physiological salt solution, was inoculated intracranially with 0.2 cubic centimeter of a suspension prepared by macerating three infective *A. ægypti* in 1.5 cubic centimeters of salt solution. The daily temperature and leukocyte reactions are shown in Table 29.

TABLE 29.—*Rabbit 2 (dark gray).*

	Days after subdural inoculation with dengue-infected mosquitces.						
	0	1	2	3	4	5	6
Temperature ____°F__	103.0	102.4	102.2	103.2	102.4	102.3	102.0
Total leukocytes______	8,100	8,200	5,900	6,100	5,600	6,500	7,500
Neutrophiles_________	1,500	4,000	2,750	1,700	1,750	1,600	2,600
Lymphocytes________	5,800	3,600	2,500	3,500	4,300	3,100	4,200
Monocytes__________	490	410	600	800	230	1,300	300

	Days after subdural inoculation with dengue-infected mosquitoes.						
	7	8	9	10	11	12	13
Temperature ____°F__	102.2	102.2	102.2	---------	102.2	103.0	101.8
Total leukocytes______	8,150	8,200	7,700	---------	8,100	9,000	9,200
Neutrophiles_________	2,600	2,400	1,600	---------	2,700	990	1,600
Lymphocytes________	5,100	5,300	5,200	---------	4,900	3,450	3,200
Monocytes__________	250	320	530	---------	320	540	280

As a control another rabbit (No. 2–A), similarly prepared, was inoculated with a suspension of normal *A. ægypti*, with the results indicated in Table 30.

TABLE 30.—*Rabbit 2–A (light gray).*

	Days after subdural inoculation with normal mosquitoes.						
	0	1	2	3	4	5	6
Temperature ____°F__	102.3	102.8	101.8	102.2	101.2	102.0	101.8
Total leukocytes______	7,800	7,600	10,200	7,400	6,200	6,200	8,700
Neutrophiles_________	1,100	1,200	810	2,400	1,600	1,200	860
Lymphocytes________	6,300	5,300	6,700	4,700	5,000	5,200	6,950
Monocytes__________	400	990	2,500	230	190	200	690

	Days after subdural inoculation with normal mosquitoes.						
	7	8	9	10	11	12	13
Temperature ____°F__	102.4	102.0	101.8	---------	101.8	102.2	101.6
Total leukocytes _____	9,700	7,900	8,100	---------	7,200	7,900	8,200
Neutrophiles_________	1,700	1,200	2,200	---------	2,000	1,200	1,200
Lymphocytes________	6,800	5,950	5,500	---------	4,900	5,900	6,650
Monocytes__________	180	545	485	---------	150	510	320

Experiment 4.—February 25, 1929. A normal rabbit (No. 3) was inoculated intracranially with 0.25 cubic centimeter of infective blood from an experimental case of dengue (A.V. 3–A). The temperature and leukocyte reactions of this rabbit are shown in Table 31.

TABLE 31.—*Rabbit 3 (red rabbit).*

	Days after subdural inoculation with 0.2 cc dengue-infected blood.											
	0	1	2	3	4	5	6	7	8	9	10	11
Temperature °F	---------	103.8	101.6	102.3	---------	103.0	103.4	102.6	103.0	102.4	102.8	102.6
Total leukocytes	15,800	12,100	15,500	15,300	---------	13,800	14,000	18,000	11,500	13,000	14,800	13,300
Neutrophiles	4,000	2,100	8,100	6,600	---------	2,500	2,550	3,500	1,900	2,200	3,300	3,100
Lymphocytes	10,700	9,400	4,300	8,000	---------	10,800	10,700	14,200	8,900	9,900	11,300	9,500
Monocytes	1,100	360	2,000	760	---------	560	850	365	450	520	455	540

Experiment 5.—May 11, 1929. Two normal rabbits (Nos. 4 and 5) were each inoculated intracranially with 0.5 cubic centimeter amounts of citrated blood from *Macacus philippinensis* 16. May 15 the rabbits were again inoculated similarly with 0.25 cubic centimeter of infective blood from an experimental case of dengue (A.V. 9–D). Rabbit 4 died immediately as a result of the inoculation. The daily temperature and leukocyte changes in rabbit 5 are shown in Table 32.

COMMENT

Subconjunctival and intracranial inoculations of dengue virus into these rabbits, which had previously lived unscreened in the City of Manila, failed to produce any specific diagnostic evidence of infection with dengue.

5. GOAT

April 16, 1929, a young male goat, obtained from the Bureau of Science farm, was inoculated intratesticularly with 0.25 cubic centimeter of infective blood from experimental case of dengue A.V. 6–R. No significant local or general reaction was noted. The rectal temperature showed no rise. The total leukocyte counts remained between 13,000 and 17,800. However, there was a marked decrease in neutrophiles on the fifth and sixth days following inoculation. (See Table 33). Comment: This animal failed to show any significant evidence of infection with dengue.

TABLE 32.—*Rabbit 5.*

	Days after subdural inoculation with 0.5 cc blood from monkey 16.									
	0	1	2	3	4	5	6	7	8	9
Temperature °F	102.6	102.2	102.6	102.0	105.0	104.0	104.0	102.8	102.0	101.8
Total leukocytes	7,900	8,550	6,550	6,800	7,350	6,950	10,150	7,400	8,050	9,300
Neutrophiles	1,975	1,282.5	931	1,836	2,058	1,598.5	2,030	518	885.5	1,023
Lymphocytes	5,927	7,267.5	5,453	4,896	5,292	5,351.5	8,120	7,084	8,277	----------

TABLE 33.—*Goat 1.*

	Days after inoculation with 0.25 cc dengue blood.									
	1	2	3	4	5	6	7	8	9	10
Temperature °F	102.2	102.2	101.8	102.0	101.8	101.0	101.6	101.2	100.0	101.0
Total leukocytes	13,900	13,550	13,000	15,500	14,350	15,650	13,450	14,900	17,850	17,850
Neutrophiles per cent	48	25	39	27	34	19	22	51	40	54
Lymphocytes do	35	63	50	65	57	78	69	38	56	41
Monocytes do	6	2	4	2	1	1	4	2	1	4

6. GUINEA PIGS

The guinea pigs used in the following experiments had previously been kept in an unscreened animal house in the City of Manila and had no doubt been bitten many times by mosquitoes.

Experiment 1.—April 16, 1929. Guinea pig 1 received an intratesticular injection of 0.25 cubic centimeter of defibrinated blood, drawn from A.V. 6–R during his first day of dengue. April 22, 1929, the animal was sacrificed and examined. There was no gross evidence of infection of the testicle and no diagnostic sign of dengue. (See Table 34).

Experiment 2.—May 15, 1929. Guinea pig 2 received intratesticularly 1 cubic centimeter of citrated blood obtained from A.V. 9–D during the first twenty-four hours of dengue fever. May 15 to May 28, no evidence of general reaction or of pathological change in the testicle. May 28, discarded. (See Table 35.) There was no indication that dengue occurred in this animal.

Experiment 3.—May 15, 1929. Guinea pig 3 received intratesticularly 1 cubic centimeter of citrated blood obtained from A.V. 9–D during the first twenty-four hours of experimental dengue fever. May 18 fed eighty-eight *Aëdes ægypti*, designated as lot 21 (see record of *A. ægypti*). (This lot of mosquitoes was tested for infectivity June 5, 1929, by feeding forty-five of them on a susceptible volunteer (A.V. 14–A), but dengue did not develop.) (See Table 35.) The guinea pig was then sacrificed and examined, but no evidence of pathology was noted. One cubic centimeter of blood from the heart was injected into the testicle of guinea pig 6. The testicle was removed, emulsified in 0.85 cubic centimeter of saline, filtered through a preliminary Mandler filter, which retained *M. melitensis*, and 0.5 cubic centimeter of the filtrate was injected subcutaneously into guinea pigs 7 and 8. No evidence was obtained to indicate that guinea pig 3 had dengue.

Experiment 4.—May 18, 1929. Guinea pig 4 was injected intratesticularly with 0.5 cubic centimeter of citrated blood from A.V. 12–S, who was in the first twenty-four hours of dengue fever. May 18 to 30, 1929, no evidence of infection. (See Table 35.) Testicle showed no enlargement. Lymph glands not palpable. May 30, 1929, discarded. There was no evidence that dengue occurred in this animal.

Experiment 5.—May 18, 1929. Guinea pig 5 was injected intratesticularly with 0.5 cubic centimeter of citrated blood obtained from A.V. 12–S during the first twenty-four hours of dengue fever. May 18 to 30, not sick. Testicles and lymph glands normal. Slight leukopenia was noted on the fifth day. May 30, discarded. This animal showed no significant evidence of infection with dengue. (See Table 35.)

Experiment 6.—May 18, 1929. Guinea pig 6 was injected intratesticularly with 1 cubic centimeter of blood from the heart of guinea pig 3. (Guinea pig 3 showed leukopenia this date and was sacrificed for examination.) May 18 to 30, no evidence of illness; testicles and lymph glands normal. (See Table 35.) May 30, discarded. This animal showed no significant evidence of dengue.

Experiment 7.—May 18, 1929. Guinea pig 7 was injected intratesticularly with 0.5 cubic centimeter of filtrate obtained by passing an emulsion of testicle from guinea pig 3 through a preliminary type of Mandler filter. May 22, 1929, normal; no change in testicles and no enlargement of inguinal lymph nodes. May 23, 1929, slight leukopenia developed, but the animal was apparently normal. (See Table 35.) Sacrificed for autopsy and to obtain material for injection into guinea pigs 11, 12, and 13. See protocols. No evidence of pathology noted. This animal showed no significant evidence of dengue.

Experiment 8.—May 18, 1929. Guinea pig 8 received an intratesticular injection of 0.5 cubic centimeter of a filtrate obtained by passing an emulsion of the testicle of guinea pig 3 through a preliminary type of Mandler filter. (Guinea pig 3 had a leukopenia May 18, but was apparently not sick.) May 19 to 30, no evidence of testicular change, enlargement of inguinal lymph nodes, or of illness; no leukopenia. (See Table 35.) May 30, discarded. This animal showed no significant evidence of dengue.

Experiment 9.—May 23, 1929. Guinea pig 9 was injected intratesticularly with 0.75 cubic centimeter of citrated blood obtained from A.V. 13–W, during the first twenty-four hours of dengue fever. May 24 to June 2, no evidence of testicular change, glandular enlargement, or of illness. (See Table 35.) June 2, discarded. This animal showed no significant evidence of dengue.

Experiment 10.—May 23, 1929. Guinea pig 10 was injected intratesticularly with 0.75 cubic centimeter of citrated blood, obtained from A.V. 13–W, during the first twenty-four hours of dengue. May 24 to June 5, no evidence of reaction in testicle or enlargement of lymph glands; there was no significant indication of illness. (See Table 35.)

Experiment 11.—May 23, 1929. Guinea pig 11 was injected intratesticularly with 0.75 cubic centimeter of blood from the heart of guinea pig 7, which showed a slight leukopenia on this date. May 24 to June 4, no evidence of illness or leukopenia (see Table 35); or of reaction in the testicle or lymph glands. June 5, discarded.

Experiment 12.—May 23, 1929. Guinea pig 12 was injected intratesticularly with 0.5 cubic centimeter of a saline emulsion of the testicle from guinea pig 7, which showed a slight leukopenia on this date but was otherwise normal. May 24 to June 5, no change in the testicle or in the lymph glands. The animal appeared well and there was no leukopenia. (See Table 35.) This animal showed no evidence of infection.

Experiment 13.—May 23, 1929. Guinea pig 13 was injected intratesticularly with 0.5 cubic centimeter of an emulsion of testicle removed from guinea pig 7. May 24 to June 5, no evidence of illness, testicular change, or of enlargement of the lymph glands. May 27, the fifth day after the inoculation, there was a decrease in leukocytes, but as this also occurred June 5, it must be discounted. This animal showed no evidence of infection.

An attempt was made to obtain nonimmune guinea pigs in the following manner: Pregnant female guinea pigs were kept in mosquito-proof cages for at least a week before they gave birth to young. The young were kept in the screened cages until used experimentally.

Experiment 14.—February 28, 1930, 11 a. m. Guinea pig 14-A, born sixteen days before and kept in a screened cage, was bitten by seven *Aëdes ægypti* of lot 135. This lot of mosquitoes had been infected December 13, 1929 (seventy-seven days previously) from A.V. 61–J. During thirteen days observation, from February 28 to March 13, there was no evidence of illness and the daily temperature records and leukocyte counts were not considered significant of infection. In order to test for a possible symptomless dengue infection, normal *Aëdes ægypti* were fed as follows: March 4, fed twenty-six; March 5, fed twenty-two; March 6, fed twenty-eight; March 7 fed thirty-two, and March 8, fed twenty-six.

These mosquitoes were combined, designated lot 175, and on March 20, 1930, one hundred two fed on a susceptible volunteer (A.V. 89–S). This volunteer did not develop dengue and he was later proved susceptible to infection by known infected mosquitoes. March 13, 1930, the guinea pig died. Comment: The feeding of dengue infected *Aëdes ægypti* on a sixteen-day-old guinea pig, which for the duration of its life had been screened from mosquitoes, did not cause infection with dengue. It was considered possible that the age of the animal, 16 days, might account for its apparent immunity.

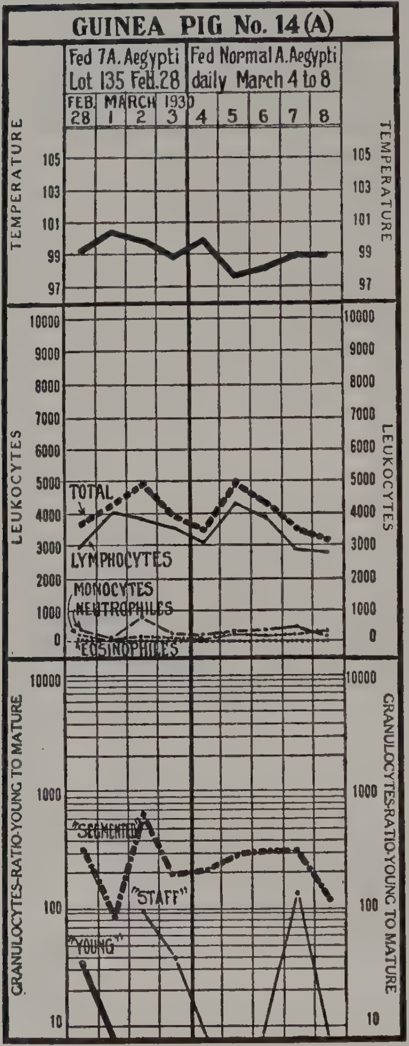

FIG. 50. Experimental dengue in guinea pig 14–A.

Experiment 15.—March 19, 1930. Guinea pig 15–B, which had been born fifteen days previously and kept in a screened cage, was bitten by twelve *A. ægypti* of lot 165. This lot of mosquitoes had been potentially infected thirty-nine days previously by feeding on A.V. 76–M, during the first day of dengue, and had subsequently transmitted dengue to A.V. 86–B. From March 19 to April 3 the guinea pig remained normal in appearance. The daily temperature records and leukocyte counts are shown in the accompanying chart (fig. 51). The latter record is suggestive of dengue infection. In order to determine the possibility of the development of a symptomless infection, normal *A. ægypti* were fed as follows: March 24, thirty-two fed; March 25, thirty-two fed; March 26, twenty-one fed, and March 27, twenty fed. These mosquitoes were combined and designated lot 187. April 23, 1930, twelve mosquitoes of this lot fed on an Ilocano volunteer, F.V. 34–R, at Baguio, Mountain Province, but dengue did not follow. The susceptibility of this volunteer was not determined, but as he had lived in the lowlands he was probably immune. April 29, 1930, four mosquitoes of lot 187 fed, and April 30 eight fed on volunteer A.V. 98–R but did not cause dengue. This volunteer was subsequently proved to be immune. Comment: The first volunteer used, F.V. 34–R, being a lowland

TABLE 34.—*Attempts to produce dengue in guinea pigs.*

Inoculation with dengue virus.					Result of inoculation.					
Guinea pig No.	Source of infective material.	Date.	Amount.	Site of injection.	Under observation.	Fever.	Leukopenia.	Other signs of illness.	Reaction at site of injection.	Conclusions.
			cc.		*Days.*					
1	A.V. 6-R	Mar. 16, 1929	0.25	Testicle	6	None	Slight, 2d day	None	None	Negative.
2	A.V. 9-D	May 15, 1929	1.0	do	12	do	None	do	do	Do.
3	A.V. 9-D	May 15, 1929	1.0	do	[a] 3	do	Yes, 2d and 3d days	do	do	(?)
4	A.V. 12-S	May 18, 1929	0.5	do	13	do	None	do	do	Negative.
5	A.V. 12-S	May 18, 1929	0.5	do	11	do	Slight, 5th day	do	do	(?)
6	Blood, guinea pig 3	May 18, 1929	1.0	do	12	do	None	do	do	Negative.
7	Testicle, guinea pig 3	May 18, 1929	0.5	do	[b] 5	do	Slight	do	do	(?)
8	do	May 18, 1929	0.5	do	11	do	None	do	do	Negative.
9	A.V. 13-W	May 23, 1929	0.75	do	9	do	do	do	do	Do.
10	A.V. 13-W	May 23, 1929	0.75	do	12	do	do	do	do	Do.
11	Blood, guinea pig 7	May 23, 1929	0.75	do	13	do	Slight, 2d day	do	do	(?)
12	Testicle, guinea pig 7	May 23, 1929	0.5	do	13	do	Slight, 2d and 3d days	do	do	(?)
13	do	May 23, 1929	0.5	do	13	do	None	do	do	Negative.

[a] Sacrificed at end of third day for transfer of material to guinea pigs 6, 7, and 8.

[b] Sacrificed the fifth day for transfer of material to guinea pigs 12 and 13.

TABLE 35.—*Total leukocytes and temperature records of guinea pigs following inoculations of dengue virus.*

Guinea pig No.	Blood count and temperature.	Days following inoculation.							
		1	2	3	4	5	6	7	8
1	Total W.B.C.	9,750	7,250	5,600	11,450	6,400	7,100	----	----
	Temperature °F	99.2	99.2	100.6	100	99	99.8	----	----
2	Total W.B.C	9,600	5,400	7,000	8,300	11,750	14,850	20,200	11,600
	Temperature °F	97.8	99.8	100.6	98.2	99.8	99.2	99.2	98.6
3	Total W.B.C.	9,400	3,900	3,650	----	----	----	----	----
	Temperature °F	102.4	101.2	102.8	----	----	----	----	----
4	Total W.B.C.	5,250	6,150	7,950	7,200	9,500	8,100	8,300	7,800
	Temperature °F	101	101	100.4	98.6	100	100.2	99.8	101
5	Total W.B.C.	8,100	6,400	8,050	7,100	5,300	5,600	9,350	9,850
	Temperature °F	98.8	100	101.8	101.4	100	100.6	99.6	101
6	Total W.B.C.	4,800	7,350	7,800	6,300	10,150	8,650	8,700	6,950
	Temperature °F	98.4	99.8	99.4	101.4	99.6	98.6	99	100.6
7	Total W.B.C.	7,250	9,450	7,900	6,850	5,050	----	----	----
	Temperature °F	100	98.8	101	100.8	100.2	----	----	----
8	Total W.B.C.	10,550	9,550	9,600	15,300	7,850	8,200	8,050	11,650
	Temperature °F	100.2	101	101.2	101	100.4	101.2	99.8	100.6
9	Total W.B.C.	10,300	10,300	11,950	10,000	9.200	8,800	8,250	6,850
	Temperature °F	100.6	99.4	101	101	100.6	101	101	99
10	Total W.B.C.	8,150	12,100	7,750	8,600	8,200	11,700	7,600	9,750
	Temperature °F	101.2	98	100.4	100.8	101.8	101.4	101.8	101.6
11	Total W.B.C.	9,300	5,150	7,200	8,650	14,800	11,800	13,600	8,350
	Temperature °F	101.8	100.8	101.6	103.4	99.6	100.6	100.8	101
12	Total W.B.C.	9,400	5,500	5,000	24,400	7,950	11,450	12,200	15,500
	Temperature °F	102.2	101.4	102	100.8	100.8	101	101	100.8
13	Total W.B.C.	16,350	13,900	12,250	9,150	6,350	9,400	8,400	9,900
	Temperature °F	100	100.2	100.2	99.2	99.8	102	101.2	100.8
14	Total W.B.C.	3,700	4,200	4,800	3,950	3,400	4,800	4,350	3,500
	Temperature °F	99.2	100.4	99.8	98.8	99.6	97.6	89.2	98.8

Guinea pig No.	Blood count and temperature.	Days following inoculation.							
		9	10	11	12	13	14	15	16
1	Total W.B.C.								
	Temperature °F								
2	Total W.B.C.	8,950	10,000	13,600					
	Temperature °F	98.6	99	97					
3	Total W.B.C.								
	Temperature °F								
4	Total W.B.C.	12,600	9,950	22,850	12,100	23,650	14,500	13,100	13,910
	Temperature °F	99	100.4	101.8	101	101	101.2	102.2	102.4
5	Total W.B.C.	13,350	7,150	11,700					
	Temperature °F	100.8	101.8	101.4					
6	Total W.B.C.	7,650	8,350	5,400					
	Temperature °F	99	99.6	101.6					
7	Total W.B.C.								
	Temperature °F								
8	Total W.B.C.	9,150	14,900	10,250					
	Temperature °F	100	101.2	101					
9	Total W.B.C.								
	Temperature °F	99.8							
10	Total W.B.C.	10,300	9,400	11,500	7,000				
	Temperature °F	102.4	100.2	100.8	98.6				
11	Total W.B.C.	8,650	9,650	7,900	11,550	8,750	11,550	11,550	
	Temperature °F	100.6	100.8	100.4	100.8	100.6	101.2	100.6	
12	Total W.B.C.	16,200	9,300	8,750	8,550	6,350	9,300	7,100	
	Temperature °F	100.4	101.4	100.4	101	102.8	101	101.4	
13	Total W.B.C.	10,800	11,350	8,100	7,800				
	Temperature °F	99.2	100.2	99.6	100.6				
14	Total W.B.C.	3,100							
	Temperature °F	98.8							

Filipino, was probably immune. The second volunteer, A.V. 98–R, was immune. Since both of the volunteers were immune, no conclusion could be drawn relative to the infection of guinea pig 15–B.

Experiment 16.—March 19, 1930. Guinea pig 16–C, that had been born in a screened cage and kept there for fifteen days, was bitten by ten known infected *A. albopictus* of lot 160. This guinea pig showed no symptoms of infection during the following two weeks.

The temperature and leukocyte counts are shown in fig. 52. The leukocyte curve suggested dengue infection. Normal A. *ægypti* were fed on this guinea pig as follows: March 24, thirty-one fed; March 25, thirty-four fed; March 26, twenty fed, and March 27, twenty fed. These mosquitoes were combined and designated lot 188. April 23, 1930, twenty-five mosquitoes of lot 188 fed on an Ilocano volunteer, F.V. 30–M, at Baguio, Mountain Province, but failed to cause dengue. The susceptibility of F.V. 30–M was never determined, but as he had lived in the lowlands, he was probably immune. April 29, six fed, and April 30, twelve mosquitoes of lot 188 fed on a susceptible volunteer, A.V. 96–H, but did not cause dengue. Comment: The first volunteer used, F.V. 30–M, being a lowland Filipino, was probably immune. While guinea pig 16–C was only fifteen days old and might have been immune to dengue, the fact that only eighteen of the mosquitoes which took blood from it later fed on the American volunteer makes it impossible to conclude that the animal was not infected.

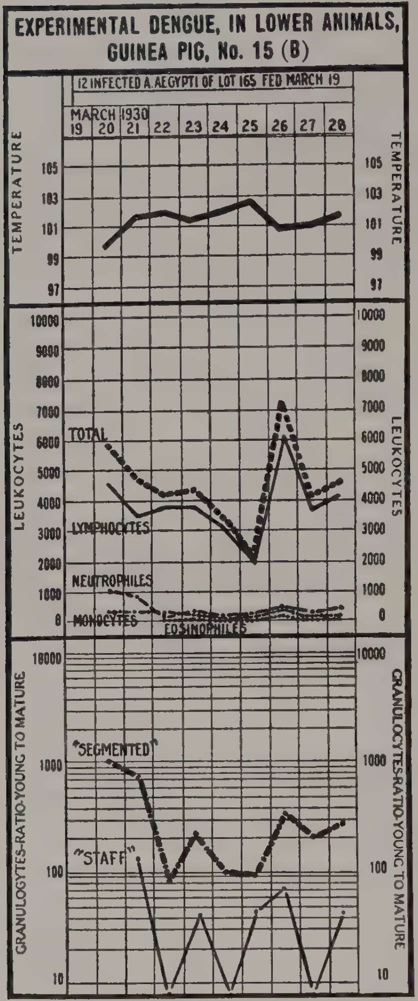

FIG. 51. Experimental dengue in guinea pig 15–B.

7. MONKEYS

The recent discovery that certain species of monkeys from nonendemic areas are susceptible to infection with the virus of yellow fever suggested the possibility that the case with dengue might be similar. Accordingly, five *Macacus rhesus,* which had lived in the National Zoölogical Park in Washington, D. C., for several years, including at least one animal born there, were obtained in May, 1928, through the courtesy of the director, Dr. W. M. Mann. These monkeys were placed in screened mosquito-proof cages and brought on an Army transport via Panama to Manila, where they arrived June 30, 1928. They were kept in screened cages at the Bureau of Science until used experimentally early in 1929. Later in 1929 arrangements were made, through Colonel Bunshiro Tanabe, M. C., Imperial Japanese Army, with an animal dealer in Tokyo for the purchase of two lots of Japanese monkeys—*Macacus fuscatus.* Five monkeys of the first shipment died en route, and the one animal that arrived safely in Manila was in an unscreened box. The four Japanese monkeys of the second shipment, which arrived several months later in good condition, were transported in screened mosquito-proof cages. As a control on the groups of *M. rhesus* (Audebert) (15) from Washington and *M. fuscatus* (Blythe) (15) from Tokyo, a large number of native Philippine monkeys, *Macacus philippinensis* S. Geoffroy (15) (*Cynomolgus philippinensis*), obtained both from endemic dengue areas, such as Manila, and from dengue-free localities

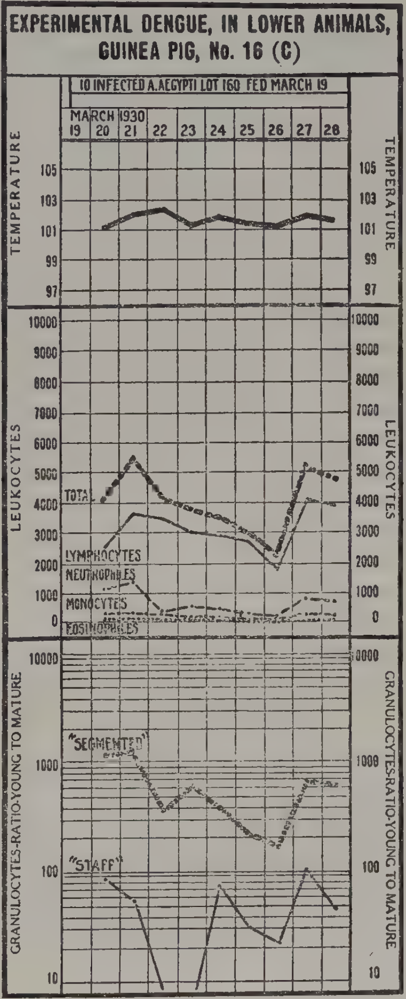

FIG. 52. Experimental dengue in guinea pig 16–C.

in the mountains of northern Luzon, were also used in transmission experiments.

Source of dengue virus.—In all of the experiments, excepting No. 13, the materials used in attempting to infect the monkeys were known to have contained dengue virus in amounts sufficient to infect man. The blood from dengue patients injected into the monkeys was usually drawn on the first day of the disease, never later than the second day; and in practically every instance its infectivity was proved by the fact that normal *A. ægypti,* fed on the patient at the time the blood was collected, later transmitted dengue to man. That the lots of mosquitoes fed on monkeys were infected was indicated by the fact that other mosquitoes of the same lots transmitted dengue to human volunteers.

Recognition of dengue infection in inoculated monkeys.—Previous studies of the striking leukocyte curves obtained in making daily counts of blood cells from a series of experimental cases of dengue in man, suggested the possibility that similar daily observations on monkeys, during the period following the inoculation of dengue virus, might serve as a means for the identification of the infection, should it occur in these animals. Consequently, in the earlier experiments with monkeys, the observations were limited almost entirely to daily studies of the physical appearance, temperature reactions, and leukocyte curves.

The temperature observations were made by rectum, usually about 11 a. m. with an ordinary clinical thermometer. Blood for the leukocyte counts was collected at the same time each day from a finger or toe. Experience in making several hundred leukocyte counts on normal monkeys indicated that a great variation is to be expected in the total leukocyte counts made at different times from the same animal. Therefore, in studying the results obtained in the transmission experiments, it was considered necessary to discount any but marked changes in the leukocyte counts. These observations were made on each animal used for a period of from two to ten days before the experimental inoculation.

In the later experiments the occurrence of infection was determined by feeding normal *Aëdes* on the monkeys each day during the period of possible infection and by subsequently testing these composite groups of mosquitoes for virus by feeding them on susceptible human volunteers.

I. OBSERVATIONS ON THE TEMPERATURE AND LEUKOCYTE REACTIONS OF MACACUS RHESUS, M. FUSCATUS, AND M. PHILIPPINENSIS, FOLLOWING THEIR INOCULATION WITH DENGUE VIRUS

MACACUS RHESUS

Experiment 1.—January 10, 1929. *Macacus rhesus* 2 from Washington, D. C., was bitten this date by four infected *A. ægypti* of lot 1, and January 11 by three more mosquitoes of the same lot. No physical signs of dengue were observed. The rectal temperature reactions were irregular. The total leukocyte count, which was 15,050 cells January 10, dropped to 10,400 January 13, then rose abruptly to 22,850 on the 14th, after which it returned to 8,000 January 17. The enormous increase in cells January 13 was due largely to a lymphocytosis, while on the other hand the neutrophiles decreased markedly January 13, 15, 16, and 17.

This monkey was tested again April 20, 1929, by the injection of 2 cubic centimeters of citrated blood from F.V. 18–P. The reaction was not significant.

Comment.—In these experiments the evidence was not considered sufficient to indicate whether or not this animal had developed dengue.

Experiment 2.—January 31, 1929. *Macacus rhesus* 3 from Washington, D. C., was bitten by fifteen infected *A. ægypti* of lot 3. No evidence of illness was recognized; both the temperature and leukocyte curves were irregular and within the normal range. Comment: In this experiment the evidence failed to indicate whether or not dengue had occurred.

Experiment 3.—January 31, 1929. *Macacus rhesus* 4 from Washington, D. C., was injected subcutaneously with 0.75 cubic centimeter of a suspension made by macerating twenty-two infected *A. ægypti* of lot 3 in 2 cubic centimeters of physiologic salt solution. There was no significant change in the daily temperature or leukocyte reactions following the injection. The lowest leukocyte count, 8,500, was obtained on the fifth day after injection.

Another attempt to infect this monkey was made April 20, 1929. With 2 cubic centimeters of citrated blood from F.V. 18–P. The reactions were not significant.

Comment.—In the experiment on this animal the evidence failed to indicate whether or not dengue had occurred.

Experiment 4.—February 28, 1929. *Macacus rhesus* 5 was brought to Manila in a screened cage from Washington, D. C., but escaped on arrival in Manila and spent one night unscreened before he was recaptured. February 28, 1929, this monkey was bitten by twelve infected *A. ægypti* of lot 3. As with the other four monkeys of this species, no significant diagnostic change was observed in the temperature or leukocyte curves. An enormous increase in neutrophilic granulocytes occurred from the fifth to the tenth day. Comment: In this experiment the evidence failed to indicate whether or not dengue had occurred.

MACACUS FUSCATUS

Experiment 5.—March 1, 1929. *Macacus fuscatus* 1, brought to Manila in an unscreened cage from Tokyo, Japan, was bitten by one infected

A. ægypti of lot 3, and again March 2 by two mosquitoes of lot 3 and by two of lot 2. This monkey showed an enormous increase in the numbers of lymphocytes, which rose from 4,770 March 1 to 12,560 March 7. Very little change was observed in the numbers of neutrophiles. Comment: This type of leukocyte reaction is the reverse of that observed during dengue in man. However, at the time of the experiment it was considered possible that it might represent a peculiar response of *M. fuscatus* to infection with dengue virus; and this led us to order another group of these monkeys from Japan for further experimental use. From later observations it is believed that the lymphocytosis observed in this monkey (No. 1) was probably due to causes other than dengue.

Note.—Later *M. fuscatus* 1 was inoculated intracardially with 2 cubic centimeters of citrated blood from a typical clinical case of dengue (F. V. 18–P). The temperature and leukocyte reactions following this inoculation were not considered significant.

MACACUS PHILIPPINENSIS

Experiment 6.—December 17, 1928 (noon). *Macacus philippinensis* 1, obtained in Manila, was injected subcutaneously with 1.5 cubic centimeters of citrated infected blood, which had just been collected from a case of dengue (Keller) during the second day of the disease. The infectivity of this blood was proved by the fact that *A. ægypti*, lot 1, which fed on Keller at the same time, later transmitted dengue to human volunteers. The monkey, however, failed to show any recognizable physical signs of infection and there was no striking change in the daily rectal temperature reactions. However, on the fourth, fifth, and sixth days after inoculation, there occurred a decrease in the number of leukocytes, particularly the neutrophilic granulocytes. This was accompanied by the appearance of from 4 to 6 per cent of the large vacuolated monocytes commonly found during or at the end of dengue in man. Comment: The decrease in leukocytes observed in this monkey occurred within the period usually required for the appearance of dengue in man; and while not proven to be diagnostic of the infection in this instance, the reaction was considered suggestive.

Experiment 7.—December 17, 1928 (noon). *Macacus philippinensis* 2, obtained in Manila, was inoculated subcutaneously with 1.5 cubic centimeters of citrated blood, which had just been collected from a dengue patient (Keller) during the second day of the disease. *Aëdes ægypti*, lot 1, fed on Keller at this time, later transmitted dengue to susceptible human volunteers. After inoculation the monkey was observed for two weeks, during which time there was no evidence of eruption or other physical signs of dengue. The daily rectal temperature fluctuations were within normal limits. However, there was a marked decrease in the leukocyte counts, from a preliminary count of 12,050 to about 5,000 cells on the fourth and fifth days after inoculation. This was due almost entirely to a decrease in the number of neutrophiles. Comment: The daily leukocyte curve of this monkey suggested the possibility that infection with dengue might have occurred about the fourth or the fifth day, but this was not proved.

Experiment 8.—January 10, 1929. *Macacus philippinensis* 4, obtained in Manila, was bitten by thirteen infected *A. ægypti* of lot 1. This animal showed no physical signs of illness. There was considerable variation in the daily leukocyte counts, but the curve was not considered indicative of dengue infection.

Experiment 9.—January 31, 1929. *Macacus philippinensis* 6, obtained in Manila, was bitten by ten infected *A. ægypti* of lot 3. The daily temperature variations were within normal limits, while the number of leukocytes increased steadily during the twelve days observation. There was no indication that this animal had developed dengue.

Experiment 10.—January 31, 1929. *Macacus philippinensis* 7, obtained in Manila, was injected subcutaneously with 0.75 cubic centimeter of a suspension prepared by macerating twenty-two infected *A. ægypti*, lot 3, in 2 cubic centimeters of physiologic salt solution. There was no elevation of temperature. The leukocyte counts dropped from 10,050 before inoculation to about 7,000 on the fifth, sixth, and seventh days, which was not considered significant.

Experiment 11.—February 28, 1929. A long-tailed monkey of unknown origin (No. 8), brought to the Philippine Islands by a sailor, was obtained in Manila from the Bureau of Science. This monkey was bitten by five infected *A. ægypti*, lot 2. There was no elevation of temperature. The leukocyte counts, which had fluctuated between 14,700 and 17,450 before inoculation, were 9,150 and 8,850 the sixth and seventh days. However, in view of the great normal daily fluctuations noted later, these observations were not considered to be significant of dengue.

Experiment 12.—February 28, 1929. A long-tailed monkey of unknown origin (No. 9), brought to the Philippine Islands several years ago by a sailor, was obtained from the Bureau of Science. This monkey was bitten by eleven infected *A. ægypti* of lot 2. No evidence of dengue was observed by physical examination or from the daily temperature reaction. The preliminary leukocyte counts varied from 12,350 to 17,650. The lowest count obtained from this monkey was 7,900 cells on the fourth day after inoculation. This was not regarded as significant.

Experiment 13.—April 20, 1929. *Macacus philippinensis* 7 was inoculated intracardially with 2 cubic centimeters of citrated blood from a typical but unproved case of dengue (F.V. 18–P). No evidence of dengue was observed in this monkey during a period of nine days. April 25, 2 cubic centimeters of blood was drawn from the heart of monkey *M. philippinensis* 7 and injected intracardially into monkey 9. May 2, seven days later, 3 cubic centimeters of citrated blood from the heart of monkey 9 was inoculated intracardially into *M. philippinensis* 14. May 7, five days later, 2 cubic centimeters of citrated blood from monkey 14 was inoculated intracardially into *M. philippinensis* 16, and May 11, 2 cubic centimeters of citrated blood from *M. philippinensis* 4 was again inoculated into monkey 16 and 2 cubic centimeters into *M. philippinensis* 17. May 11, 2 cubic centimeters of citrated blood from the heart of monkey 16 was injected intrapleurally into *M. philippinensis* 18. The six monkeys used in this series of tests all failed to show any significant indication of dengue infection.

Experiment 14.—April 29, 1929. *Macacus philippinensis* 10 and 11, obtained in Manila, were each inoculated intracardially with 2 cubic centimeters of infective blood, which had just been collected from an experimental case of dengue (A.V. 10–H) during the first day of the disease. There was no apparent change in the physical appearance of this monkey. However, the temperature of monkey 11 reached 104° F. on the fifth day, and on the sixth day the neutrophilic granulocytes and lymphocytes of this monkey were markedly reduced. Monkey 11 showed nothing of significance with the possible exception of a reduction in neutrophiles after the fourth day. These reactions were not considered diagnostic.

Experiment 15.—May 1, 1929. *Macacus philippinensis* 12 and 13, obtained in Manila, were each injected intracardially with 5.5 cubic centimeter amounts of citrated blood immediately after its collection from an experimental case of dengue (A.V. 8–G), during the first day of the disease. The daily temperature and leukocyte reactions failed to show any significant changes.

Experiment 16.—May 14, 1929. *Macacus philippinensis* 20 and 21, both very young, were obtained in Manila. Each was injected subcutaneously with 2 cubic centimeters of citrated blood drawn from an experimental case of dengue (A.V. 9–D) during the first day of the disease. May 15, each received an additional 2 cubic centimeters of citrated blood from (A.V. 9–D) subcutaneously. Neither showed any physical evidence of illness, and the temperature and leukocyte counts were irregular. The lowest count for monkey 20 was 11,500, obtained the fourth day; and the lowest for monkey 21 was 7,600, obtained the same day. There was no recognizable sign of dengue.

Experiment 17.—May 19, 1929. *Macacus philippinensis* 19 was inoculated intracardially with 3 cubic centimeters of citrated blood, collected from the heart of *M. philippinensis* 21, the fifth day after that monkey had been inoculated with infected blood. No significant change was noted in the appearance of No. 19 or in the temperature or leukocyte counts.

Practically all of the monkeys used in these seventeen experiments were later reinoculated with materials known to contain dengue virus. The results were not significant, and the variations in temperature and leukocyte counts were irregular, as in the initial tests.

COMMENT

1. In these experiments twenty-six monkeys, including four *Macacus rhesus* from Washington, D. C., one *M. fuscatus* from Tokyo, and twenty-one *M. philippinensis* from an endemic dengue area (Manila), were inoculated with infective blood from experimental dengue-fever patients or from monkeys showing some slight indication of dengue infection, or with mosquitoes containing dengue virus, or were bitten by dengue-infected mosquitoes.

2. Before and after the inoculations these animals were observed daily for physical signs of illness, and daily records were made of the rectal temperature and of the leukocyte counts.

3. While some of the inoculated animals showed a decrease in the numbers of leukocytes that was suggestive, these changes

were not sufficiently striking or constant to be considered as diagnostic of infection with dengue.

II. DENGUE PRODUCED IN MONKEYS AND PROVED BY RETRANSFER OF THE INFECTION THROUGH MOSQUITOES TO MAN [4]

The failure of the monkeys to show typical physical signs and the leukopenia observed during dengue in man seemed to indicate either that they were completely immune to dengue or that the infection was manifested in some way not recognizable by our examinations. However, as a few of the animals, for example, *Macacus philippinensis* 1 and 2, had blood counts that were at least suggestive of a leukopenia on the fourth, fifth, or sixth day after inoculation; and as this corresponds roughly with the incubation period in man, it was decided to test the animals for infectivity, regardless of symptoms, by feeding normal mosquitoes on them during the fifth or sixth day after inoculation, and later allowing these mosquitoes to feed on man.

EXPERIMENTS IN WHICH INOCULATED MONKEYS WERE TESTED FOR DENGUE VIRUS ON EITHER THE FIFTH OR THE SIXTH DAY

MACACUS RHESUS

Experiment 1.—January 9, 1929. *Macacus rhesus* 1, brought to Manila in a screened cage from Washington, D. C., was inoculated subcutaneously with 1 cubic centimeter of citrated blood, which had just been drawn from an experimental dengue patient (A.V. 2–M), during the second day of the disease. This monkey showed no recognizable physical signs of dengue. However, the leukocyte curve showed some depression below the level of the preliminary counts made before inoculation. This was particularly true of the neutrophilic granulocytes, which were decreased in numbers on the fourth, fifth, and sixth days following the inoculation; while the numbers of lymphocytes increased steadily from about 3,500 cells January 9 to 10,000 January 16.

Attempt to infect normal mosquitoes.—January 15, or the sixth day after inoculation, fourteen normal *A. ægypti* took blood from this monkey and were designated lot 4. Ten mosquitoes of this lot were later combined with two of lot 5, and nine of lot 6, both of which had fed on other inoculated monkeys.

Test of mosquitoes for infectivity.—February 12 all the mosquitoes in the combined lots fed on a susceptible volunteer (A.V. 3–A), but dengue did not result.

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[4] During the progress of these studies Blanc et al. (14) (1929) reported that they were successful in transmitting dengue virus from man to two species of monkeys (*Cynomolgus fascicularia* and *Cercopithicus callitricus*) and back to man by blood inoculations. They were unable to transmit the infection from monkey to monkey. They apparently did not attempt to transmit dengue from monkey to man by mosquitoes.

TABLE 36.—*Total leukocytes and temperature records of monkeys following inoculations of dengue virus.*

Experiment No.	Monkey No.	Date of experiment.	White blood count and temperature.	Days following inoculation.								
				0	1	2	3	4	5	6	7	8
1 (a)	M.R. 2	Jan. 10, 1929	Total W.B.C.	15,050	15,600	12,450	10,400	22,850	12,650	11,800	8,100	11,700
			Temperature °F	101.0	100.1	99.9	100.2	100.1	100.1	101.1	100.8	101.0
				102.4	102.0	102.2	------	------	100.0	101.4	100.0	99.2
1 (b)	M.R. 2	Apr. 20, 1929	Total W.B.C.	------	------	10,800	14,100	9,500	13,050	8,100	9,450	10,900
			Temperature °F	------	------	100.8	100.8	101.6	101.6	100.8	102.8	101.6
2 (a)	M.R. 3	Jan. 31, 1929	Total W.B.C.	14,450	10,650	10,250	12,100	10,100	8,950	7,350	8,700	10,100
			Temperature °F	100.1	100.2	101.6	99.9	101.2	100.4	101.2	101.0	101.2
				------	100.6	100.4	------	101.6	100.6	99.8	100.4	99.4
2 (b)	M.R. 3	Apr. 20, 1929	Total W.B.C.	------	------	7,250	8,600	8,550	8,850	8,750	9,350	7,750
			Temperature °F	------	------	102.0	101.2	103.0	102.6	102.6	103.4	102.6
3	M.R. 4	Jan. 31, 1929	Total W.B.C.	35,650	17,450	11,300	13,450	10,400	8,500	12,750	9,050	9,200
			Temperature °F	102.0	101.2	100.2	101.6	102.2	102.1	101.4	101.6	101.8
				------	100.6	100.8	------	101.8	101.8	100.6	100.8	99.6
4	M.R. 5	Feb. 28, 1929	Total W.B.C.	12,100	11,100	10,200	13,150	9,400	16,350	22,100	28,950	29,800
			Temperature °F	99.8	99.2	99.4	100.0	99.4	99.0	99.4	101.4	100.8
5 (a)	M.F. 1	Mar. 1, 1929	Total W.B.C.	9,900	8,150	9,150	12,850	13,100	15,850	15,900	11,150	12,200
			Temperature °F	100.8	102.6	101.2	102.2	101.2	101.8	101.8	101.6	101.0
5 (b)	M.F. 1	Apr. 20, 1929	Total W.B.C.	------	------	12,150	5,150	9,350	7,850	7,100	6,650	5,350
			Temperature °F	------	------	102.0	102.0	102.4	102.2	102.0	103.0	102.4
6 (a)	M.P. 1	Dec. 17, 1928	Total W.B.C.	------	8,400	7,950	8,150	6,500	5,200	6,500	6,550	6,700
			Temperature °F	------	99.8	99.1	98.9	98.8	99.2	100.1	99.0	98.9
B-2	M.P. 3	Jan. 9, 1929	Total W.B.C.	10,850	9,100	13,300	11,050	8,900	9,250	7,350	6,850	6,250
			Temperature °F	99.6	98.6	99.8	99.8	99.8	98.9	99.1	99.2	98.9
				------	99.8	99.0	99.4	------	------	99.2	99.0	101.6
7 (a)	M.P. 2	Dec. 17, 1928	Total W.B.C.	12,050	11,900	9,100	8,650	5,050	5,150	10,100	8,650	7,900
			Temperature °F	------	99.9	100.0	99.8	99.1	99.9	101.6	98.6	99.1

B-3	M.P. 5	Jan. 10, 1929	Total W.B.C.	15,600	18,950	16,200	9,850	22,250	12,100	12,400	6,350	4,350
			Temperature °F	97.2 100.2	99.9 99.6	99.2 102.2	98.9 --------	99.1 --------	98.7 100.0	98.8 99.2	98.4 99.2	98.8 97.8
8	M.P. 4	Jan. 10, 1929	Total W.B.C.	11,050	12,850	14,950	7,300	12,450	7,150	7,100	7,800	9,250
			Temperature °F	99.1 100.0	98.9 100.0	100.2 99.8	98.8 --------	99.1 --------	98.9 99.4	98.9 99.8	98.8 100.2	98.4 99.8
9	M.P. 6	Jan. 31, 1929	Total W.B.C.	32,300	7,700	10,100	6,950	10,850	15,250	13,950	17,450	13,850
			Temperature °F	99.1 --------	98.9 100.0	98.3 99.8	98.4 --------	99.2 99.8	98.8 98.6	98.8 99.2	98.6 99.4	98.8 99.4
10	M.P. 7	Jan. 31, 1929	Total W.B.C.	10,050	12,150	7,850	9,850	8,900	7,150	7,850	7,050	9,150
			Temperature °F	98.8 --------	98.4 99.4	98.6 99.2	100.0 --------	98.4 99.2	98.4 98.8	98.2 99.4	98.0 99.0	98.4 99.2
11	M.P. 8	Feb. 28, 1929	Total W.B.C.	17,450	10,950	12,100	15,050	11,800	12,400	9,150	8,850	13,100
			Temperature °F	101.0	101.8	101.4	99.8	99.8	100.8	99.2	100.2	100.2
12	M.P. 9	Feb. 28, 1929	Total W.B.C.	12,350	15,450	13,850	14,350	7,900	14,450	10,800	10,250	16,850
			Temperature °F	102.6	98.8	100.2	99.8	100.2	101.2	101.0	101.2	101.2
13 "A"	M.P. 7	Apr. 20, 1929	Total W.B.C.	--------	--------	12,150	6,850	7,800	8,200	7,950	7,800	6,400
			Temperature °F	--------	--------	101.8	101.8	101.0	100.0	100.8	101.8	102.2
13 "B"	M.P. 9	Apr. 25, 1929	Total W.B.C.	15,450	15,400	18,350	13,900	14,050	12,400	10,300	9,500	10,600
			Temperature °F	101.4	101.4	100.8	100.6	101.0	100.8	101.2	101.0	101.0
14 "A"	M.P. 10	Apr. 29, 1929	Total W.B.C.	--------	19,700	23,200	18,100	14,950	13,000	11,950	18,250	12,200
			Temperature °F	--------	100.8	101.4	101.6	102.8	102.6	102.2	102.2	102.0
14 "B"	M.P. 11	Apr. 29, 1929	Total W.B.C.	--------	15,650	23,450	12,350	15,800	11,500	10,350	9,600	11,900
			Temperature °F	--------	101.2	102.4	102.6	103.4	103.6	104.0	103.2	102.4
15 "A"	M.P. 12	May 1, 1929	Total W.B.C.	--------	8,050	14,350	9,800	12,450	14,050	10,350	10,300	11,300
			Temperature °F	--------	98.2	100.4	99.8	101.2	100.2	99.2	100.0	99.2
15 "B"	M.P. 13	May 1, 1929	Total W.B.C.	--------	10,150	18,300	14,650	15,100	19,650	14,400	15,650	15,450
			Temperature °F	--------	101.2	100.4	101.0	101.4	101.0	101.2	100.2	101.4
13 "C"	M.P. 14	May 2, 1929	Total W.B.C.	--------	11,200	11,900	13,400	10,700	7,900	10,850	9,600	8,300
			Temperature °F	--------	102.8	102.2	102.4	101.6	101.6	102.0	101.4	100.6
13 "D"	M.P. 16	May 7, 1929	Total W.B.C.	--------	13,100	15,550	8,400	11,000	11,950	10,950	10,650	13,200
			Temperature °F	--------	101.4	102.0	101.8	101.0	101.0	101.6	101.6	101.0
13 "E"	M.P. 17	May 11, 1929	Total W.B.C.	12,650	13,900	11,250	15,400	10,300	16,600	11,100	12,350	11,800
			Temperature °F	102.0	100.6	101.2	101.8	102.0	101.4	101.6	102.0	102.2

TABLE 36.—*Total leukocytes and temperature records of monkeys following inoculations of dengue virus*—Continued.

Experiment No.	Monkey No.	Date of experiment.	White blood count and temperature.	Days following inoculation. 0	1	2	3	4	5	6	7	8
13 "F"	M.P. 18	May 11, 1929	Total W.B.C.	15,850	21,450	20,800	20,800	30,700	22,600	17,150	--------	--------
			Temperature °F	102.2	102.4	102.0	103.0	102.8	102.6	103.2	--------	--------
17	M.P. 19	May 19, 1929	Total W.B.C.	--------	16,650	12,300	12,550	16,750	10,800	8,100	9,700	13,100
			Temperature °F	--------	101.8	100.8	100.6	100.2	100.0	100.4	100.0	100.0
16 "A"	M.P. 20	May 14, 1929	Total W.B.C.	--------	24,300	15,100	13,500	11,950	11,400	16,650	16,700	12,400
			Temperature °F	--------	101.0	101.4	100.2	102.6	102.2	103.0	102.4	101.6
16 "B"	M.P. 21	May 14, 1929	Total W.B.C.	12,950	9,900	14,600	11,550	7,750	9,650	12,400	9,200	12,000
			Temperature °F	102.2	101.8	101.4	101.6	101.0	101.8	101.6	102.2	101.4

Experiment No.	Monkey No.	Date of experiment.	White blood count and temperature.	Days following inoculation. 9	10	11	12	13	14	15	16
1 (a)	M.R. 2	Jan. 10, 1929	Total W.B.C.	11,600	--------	--------	--------	--------	--------	--------	--------
			Temperature °F	100.8	--------	--------	--------	--------	--------	--------	--------
1 (b)	M.R. 2	Apr. 20, 1929	Total W.B.C.	13,000	10,300	10,400	--------	--------	--------	--------	--------
			Temperature °F	101.8	101.0	102.0	--------	--------	--------	--------	--------
2 (a)	M.R. 3	Jan. 31, 1929	Total W.B.C.	8,800	--------	8,050	--------	--------	--------	--------	--------
			Temperature °F	100.8	--------	101.4	--------	--------	--------	--------	--------
2 (b)	M.R. 3	Apr. 20, 1929	Total W.B.C.	7,000	6,850	7,700	--------	--------	--------	--------	--------
			Temperature °F	103.0	102.4	102.2	--------	--------	--------	--------	--------
3	M.R 4	Jan. 31, 1929	Total W.B.C.	10,400	--------	10,900	--------	--------	--------	--------	--------
			Temperature °F	101.2	--------	102.2	--------	--------	--------	--------	--------

4	M.R. 5	Feb. 28, 1929	Total W.B.C.	31,050	29,050	22,250	26,050	33,600	-	-	-
			Temperature °F	101.8	100.8	98.8	98.4	98.2	-	-	-
5 (a)	M.F.1	Mar. 1, 1929	Total W.B.C.	10,800	11,400	16,150	14,850	12,650	9,850	10,100	9,650
			Temperature °F	100.2	101.4	99.0	99.2	99.2	100.2	99.2	99.0
5 (b)	M.F. 1	Apr. 20, 1929	Total W.B.C.	7,850	9,050	9,550	12,900	10,500	12,900	12,450	12,550
			Temperature °F	102.8	102.2	102.0	102.0	102.2	102.4	102.4	102.0
6 (a)	M.P. 1	Dec. 17, 1928	Total W.B.C.	6,250	6,900	9,000	7,650	-	-	-	-
			Temperature °F	100.1	99.2	99.2	99.1	-	-	-	-
B–2	M.P. 3	Jan. 9, 1929	Total W.B.C.	17,850	25,450	-	-	-	-	-	-
			Temperature °F	99.3 98.4	98.8	-	-	-	-	-	-
7 (a)	M.P. 2	Dec. 17, 1928	Total W.B.C.	8,300	8,100	7,700	6,950	-	-	-	-
			Temperature °F	99.2	99.4	100.4	100.3	-	-	-	-
B–3	M.P. 5	Jan. 10, 1929	Total W.B.C.	8,600	-	-	-	-	-	-	-
			Temperature °F	99.2	-	-	-	-	-	-	-
8	M.P. 4	Jan. 10, 1929	Total W.B.C.	11,250	-	-	-	-	-	-	-
			Temperature °F	98.6	-	-	-	-	-	-	-
9	M.P. 6	Jan. 31, 1929	Total W.B.C.	17,150	-	13,450	-	-	-	-	-
			Temperature °F	101.0	-	100.8	-	-	-	-	-
10	M.P. 7	Jan. 31, 1929	Total W.B.C.	6,850	-	7,150	-	-	-	-	-
			Temperature °F	99.2	-	98.6	-	-	-	-	-
11	M.P. 8	Feb. 28, 1929	Total W.B.C.	11,450	12,250	8,800	8,650	11,300	12,150	12,800	-
			Temperature °F	99.0	100.2	99.2	99.0	98.8	99.0	101.0	-
12	M.P. 9	Feb. 28, 1929	Total W.B.C.	15,100	14,100	18,650	22,550	8,800	-	-	-
			Temperature °F	99.4	100.6	102.4	102.0	102.2	-	-	-
13 "A"	M.P. 7	Apr. 20, 1929	Total W.B.C.	7,450	7,900	10,250	10,750	14,250	19,700	23,400	29,950
			Temperature °F	102.0	101.4	101.8	101.4	101.8	102.6	102.8	102.6
13 "B"	M.P. 9	Apr. 25, 1929	Total W.B.C.	16,050	10,000	14,050	14,250	15,050	16,600	-	-
			Temperature °F	101.8	101.4	101.0	100.0	101.4	101.8	-	-

TABLE 36.—*Total leukocytes and temperature records of monkeys following inoculations of dengue virus*—Continued.

Experiment No.	Monkey No.	Date of experiment.	White blood count and temperature.	Days following inoculation.							
				9	10	11	12	13	14	15	16
14 "A"	M.P. 10	Apr. 29, 1929	Total W.B.C.	11,850	11,350						
			Temperature °F	101.6	102.4						
14 "B"	M.P. 11	Apr. 29, 1929	Total W.B.C.	9,000	10,600						
			Temperature °F	103.6	103.0						
15 "A"	M.P. 12	May 1, 1929	Total W.B.C.	9,400	12,400	9,200	10,300	16,250			
			Temperature °F	99.6	99.6	99.0	98.6	98.8			
15 "B"	M.P. 13	May 1, 1929	Total W.B.C.	20,500	15,600	13,600	12,200	11,400			
			Temperature °F	99.4	100.4	100.0	100.2	99.6			
13 "C"	M.P. 14	May 2, 1929	Total W.B.C.	7,850	7,650	9,350	11,750	13,350	9,750	10,900	8,650
			Temperature °F	101.6	101.0	101.0	101.4	100.2	101.6	102.0	101.0
13 "D"	M.P. 16	May 7, 1929	Total W.B.C.	17,400	16,250	14,850					
			Temperature °F	101.8	101.4	101.4					
13 "E"	M.P. 17	May 11, 1929	Total W.B.C.	12,900	19,050	22,200					
			Temperature °F	100.6	102.0	101.8					
13 "F"	M.P. 18	May 11, 1929	Total W.B.C								
			Temperature °F								
17	M.P. 19	May 19, 1929	Total W.B.C.	11,250	8,800						
			Temperature °F	100.4	101.2						
16 "A"	M.P. 20	May 14, 1929	Total W.B.C.	16,150	19,700						
			Temperature °F	101.8	101.4						
16 "B"	M.P. 21	May 14, 1929	Total W.B.C.	12,500							
			Temperature °F	101.0							

Comment.—If dengue occurred in this monkey, the infection was not demonstrable by our observations; and on the sixth day the virus was not present in sufficient concentration to infect *A. ægypti*, lot 4. It is, of course, possible that had normal mosquitoes been allowed to feed during other days they might have become infected.

MACACUS PHILIPPINENSIS

Experiment 2.—January 9, 1928. *Macacus philippinensis* 3 (a), obtained from the Bureau of Science in Manila, was injected subcutaneously with 1 cubic centimeter of citrated blood, collected one hour previously from an experimental case of dengue (A.V. 2–M), during the second day of the disease. The rectal temperature remained normal for ten days. However, the leukocyte curve showed some depression on the fifth, sixth, and seventh days.

Attempt to infect normal mosquitoes.—January 15, or the sixth day after the inoculation, five normal *A. ægypti* were allowed to feed on this monkey, and were designated lot 5.

Test of mosquitoes for infectivity.—February 12 two of the lot 5 mosquitoes were combined with ten and nine mosquitoes, respectively, of lots 4 and 6, both of which had fed on other inoculated monkeys, and were tested on a susceptible human volunteer (A.V. 3–A). Dengue did not result from the feeding of these mosquitoes.

Comment.—Although this monkey showed no clinical evidence of dengue, and the normal mosquitoes fed on it during the sixth day after inoculation did not become infected, it is still possible that dengue occurred and that had mosquitoes been fed on other days they might have become infected.

Experiment 3.—January 10, 1929. *Macacus philippinensis* 5 (c), obtained from the Bureau of Science in Manila, was given a subcutaneous injection of 1 cubic centimeter of citrated blood drawn from an experimental case (A.V. 1–H) on the first day of the disease. No evidence of dengue was noted.

Attempt to infect normal mosquitoes.—January 15, five days later, fifteen normal *A. ægypti* were fed on the monkey and designated lot 6.

Test of mosquitoes for infectivity.—February 12 nine of these mosquitoes, with two of lot 5 and ten of lot 4, were tested by their feeding on a susceptible human volunteer (A.V. 3–A), who failed to develop dengue.

Comment.—In this experiment there was no significant clinical indication of dengue; and normal *A. ægypti* fed on the monkey on the fifth day failed to transmit the disease. However, it is possible that if normal mosquitoes had fed during other days they might have become infected.

Experiment 4.—April 14, 1930. *Macacus philippinensis* 33 (Sagada D) that had been caught in a nonendemic area in the mountains of northern Luzon, shipped to Manila in a screened box, and observed in Manila for ten days in a screened cage, was bitten by eighteen known-infected *A. albopictus* of lot 183. This monkey showed no signs of dengue.

Attempts to infect normal A. ægypti.—April 20, six days later, one hundred fifty-five normal *A. ægypti* fed on this monkey and were designated lot 214. Immediately after the mosquitoes of lot 214 had completed their feeding, the monkey was autopsied, and the liver, spleen, and brain were removed. Forty-eight normal *A. ægypti* were fed from a feeding cell on 0.5 gram of the spleen emulsified in 2 cubic centimeters of ascitic fluid and 2 cubic centimeters of normal defibrinated human blood, and were designated lot 219. One hundred thirteen normal *A. ægypti* fed from a feeding cell on 0.5 gram of the liver emulsified in 2 cubic centimeters of ascitic fluid and 2 cubic centimeters of defibrinated normal human blood, and were designated lot 220. One hundred two normal *A. ægypti* fed from a feeding cell on 0.5 gram of the brain from monkey 33 and 0.5 gram of brain from monkey 34 emulsified in 2 cubic centimeters of ascitic fluid and 2 cubic centimeters of defibrinated normal human blood and were designated lot 218.

Test of mosquitoes for infectivity.—(*a*) May 10, 1930, seventy-six mosquitoes of lot 214 fed on susceptible volunteer A.V. 96–H, but did not cause dengue. (*b*) May 10, thirteen mosquitoes of lot 219 and thirty-nine mosquitoes of lot 220 fed on susceptible volunteer A.V. 97–H, but did not cause dengue. (*c*) See experiment 5 for testing of lot 218.

Comment.—The blood, brain, liver, and spleen of *Macacus philippinensis* 33–D were shown not to be infected with dengue virus on the sixth day following the feeding of known-infective mosquitoes on the monkey.

Experiment 5.—April 14, 1930. *Macacus philippinensis* 34 (Sagada E), that had been caught in a nonendemic area and kept screened subsequently, was bitten by nine known-dengue-infected *A. albopictus*, lot 183. No symptoms of dengue were observed in this monkey.

Attempts to infect normal A. ægypti.—April 20. This monkey was treated in the same manner as described for *M. philippinensis* 33 in experiment 4 above. Sixty-five normal *A. ægypti* fed from the living monkey and were designated lot 215. One hundred nine normal *A. ægypti* fed on spleen-ascitic-fluid-blood emulsion and were designated lot 216. Twenty-three normal *A. ægypti* fed on liver-ascitic fluid emulsion and were designated lot 217. One hundred two normal *A. ægypti* fed from a brain-ascitic-fluid-blood emulsion and were designated lot 218.

Test of mosquitoes for infectivity.—(*a*) May 10, twenty-four mosquitoes of lot 215 fed on A.V. 98–R, but did not cause dengue. This volunteer was later found to be immune to dengue. (*b*) May 10, thirty-six mosquitoes of lot 216, five from lot 217, and forty-nine from lot 218 fed on susceptible volunteer A.V. 95–D, but did not cause dengue.

Comment.—*Macacus philippinensis* 34–E apparently did not contain transmissible dengue virus in the organs or blood on the sixth day following the potential infection of this monkey.

Because of the failure to demonstrate infection in these five experiments, in which attempts were made to infect mosquitoes from monkeys on the fifth or sixth days only after inoculation, at which time there was usually some decrease in the numbers of leukocytes, it was considered possible that the time selected might have corresponded with the period of extreme leukopenia observed in man, which is often too late in the disease for the infection of mosquitoes. It was also considered possible that the mosquitoes might have been fed too soon. Consequently, the following experiments were planned, in which monkeys were inoculated with dengue virus and were subsequently tested for infection by feeding different groups of normal mosquitoes on them daily between the third and the ninth days. The various groups of mosquitoes were then combined and tested for infectivity by feeding them later on susceptible volunteers.

EXPERIMENTS IN WHICH MONKEYS WERE TESTED FOR DENGUE VIRUS ON THE THIRD, FOURTH, FIFTH, SIXTH, SEVENTH, AND EIGHTH DAYS AFTER INOCULATION WITH INFECTIVE MATERIALS

MACACUS FUSCATUS

Experiment 1.—October 7, 1929 (noon). *Macacus fuscatus* 2 (W), which had been shipped from Tokyo, Japan, in a screened cage, was received in Manila September 27, 1929, and observed in a mosquito-proof cage at the Bureau of Science for ten days. October 7 this monkey was bitten by forty-four dengue-infected *A. ægypti* of lot 52, which had taken blood from an experimental case of dengue (A.V. 31–D) at 11.45 a. m., August 31, during the first day of the disease; and had been proved infected by the transmission of dengue to several susceptible human volunteers (A.V. 46–L, A.V. 44–F, A.V. 41–F, and A.V. 43–D). Daily observations were made of the monkey for a period of one month, but typical physical signs of dengue were not observed. There was some elevation of the rectal temperature from the fourth to the eighth day after infection, but as the temperature was also elevated at intervals during the period of subsequent observation, its significance remains in doubt. The total leukocyte counts, which had varied from a low of 13,600 to a high of 20,600 during the period of normal observation, dropped to 9,850, 10,050, 9,300, and 10,800 on the third, fourth, fifth, and sixth days, respectively, following the infective biting, and after a temporary rise to 17,300 on the seventh day returned to 10,300 on the eighth. This change in the total numbers of leukocytes was due to roughly similar changes in both the neutrophilic granulocytes and in the lymphocytes. On the fifth and sixth days large vacuolated monocytes, such as are frequently seen in the blood of human cases of dengue were noted. However, there was no definite leukopenia or other diagnostic evidence of dengue.

Transfer of virus from the monkey to normal Aëdes.—Beginning October 10, or the third day after the infective biting, different groups of thirty normal *A. ægypti* each were given an opportunity to feed on the monkey daily until October 15. The numbers and designations assigned to the mosquitoes that took blood from the monkey on the different days were as follows: Third day, thirty mosquitoes called lot 82; fourth day, 28 mosquitoes called lot 83; fifth day, twenty-nine mosquitoes called lot 84; sixth day, twenty-four mosquitoes called lot 85; seventh day, twenty-eight mosquitoes called lot 88; and eighth day, twenty-seven mosquitoes called lot 89.

Tests of mosquitoes for infectivity.—Test (*a*). October 28, or thirteen days after the last lot had fed on the monkey, the mosquitoes remaining alive in the various lots were all given an opportunity to feed on a single normal susceptible volunteer (A.V. 53–C), who had previously been ob-

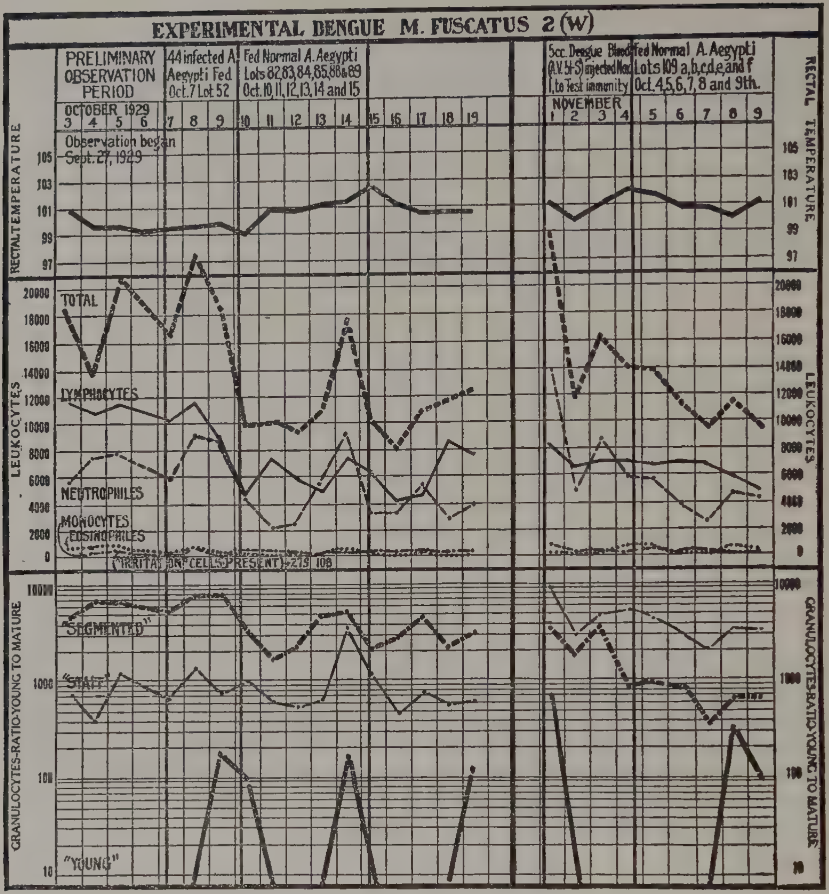


FIG. 53. Experimental dengue in *Macacus fuscatus* 2–W.

served in a screened cubicle for ten days. Six, fifteen, four, six, fifteen, and nine mosquitoes, respectively, of the various lots took blood from this volunteer who after an incubation period of seven days, developed typical dengue fever November 4 (see fig. 54).

Test (*b*).—November 5, 1929, or at least twenty-one days after having taken blood from monkey 2 (W), six, twelve, three, four, twelve, and ten mosquitoes, respectively, of those remaining alive in lots 82 to 89, were combined and given an opportunity to bite a normal Japanese monkey, *M. fuscatus* 4 (Y). Twenty-seven of the forty-seven mosquitoes took blood, and in this way transmitted dengue to the second monkey as is described in experiment 3.

Test (*c*).—November 23, 1929, or at least thirty-nine days after the feeding on *M. fuscatus* 2,-W, an attempt was made to again transfer dengue with eleven mosquitoes of the combined lots (82 to 89) to a volunteer (A.V. 58–S). However, this volunteer was later shown to be immune.

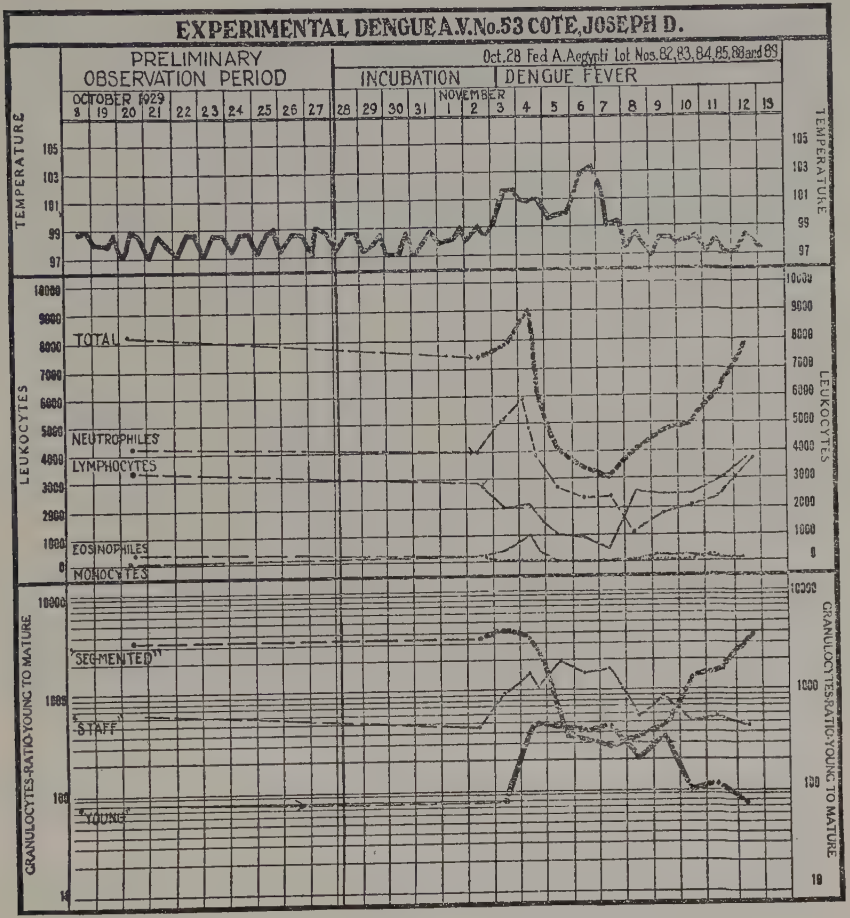


Fig. 54. **Experimental dengue in American volunteer 53.**

Later test of M. fuscatus 2 (W) for immunity to dengue.—November 1, *M. fuscatus* 2 (W) was injected intravenously with 5 cubic centimeters of warm citrated infective blood, which had just been drawn from an experimental case of dengue (A. V. 51–S), during the first few hours of the disease. Blood from the same source produced dengue in *M. fuscatus* 3 (X), as described in experiment 2. However, monkey 2 (W) showed no clinical signs indicative of dengue following this inoculation. Five different groups of thirty normal *A. ægypti* were given an opportunity to feed on the monkey each day between November 5 and 9, and the thirty, thirty, thirty, twenty-six, and twenty-eight mosquitoes which took blood on these days were designated lots 109–A, B, C, D, and E, respectively. Before being tested for infectivity, twenty-four, twenty-six, twenty-seven, twenty-four, and twenty-four mosquitoes of the various lots were placed in a single cage November 27. The following day seventy-seven mosquitoes of this combined lot (109) fed on a susceptible volunteer (A.V. 61–J). The fact that dengue did not develop from this procedure indicates that the second attempt to infect *M. fuscatus* 2 (W) failed, due, no doubt, to the immunity produced by the experimental infection, two weeks earlier.

Experiment 2.—November 1, 1929. *Macacus fuscatus* 3 (X), which had been kept in mosquito-proof cages since its departure from Tokyo, Japan, was inoculated subcutaneously, at several points over the chest and the abdomen, with 5 cubic centimeters of warm citrated infective blood, which had just been collected from an experimental dengue patient (A.V. 51–S), during the first few hours of dengue. This monkey did not show any recognizable evidence of dengue during a period of four weeks.

Transfer of virus from the monkey to normal Aëdes.—Between November 5—9, daily feedings of twenty, twenty-seven, thirty, twenty-eight, and twenty normal *A. ægypti* took place on the monkey and these different lots were called 110–A, B, C, D, and E, respectively. November 27 the different lots, including twenty-two of "A," twenty-eight of "B," nineteen of "C," twenty-seven of "D," and twenty-seven of "E," were combined in one cage and designated lot 110.

Test of mosquitoes for infectivity.—November 28, at 8.15 a. m., seventy-five *A. ægypti* of lot 110 fed on a susceptible volunteer (A.V. 62–H). After an incubation period of six days this volunteer developed typical dengue fever, beginning December 4. The diagnosis in this case (A.V. 62–H) was further substantiated by the fact that normal mosquitoes fed at this time, and designated *A. albopictus* lot 128, later transmitted the infection to two volunteers (A.V. 69–K and A.V. 72–E) and also to two monkeys *M. philippinensis* 30 (Sagada A), and 31 (Sagada B).

Comment.—The Japanese monkey, *M. fuscatus* 3 (X), was infected with dengue by an injection of infective human blood and the virus was transferred through mosquitoes to man.

Experiment 3.—November 5, 1929. *Macacus fuscatus* 4 (Y), which had been protected from mosquitoes in screened cages since its departure from Tokyo, Japan, was bitten by twenty-seven *A. ægypti* of the combined lots 82, 83, 84, 85, 88, and 89, which had been infected from *M. fuscatus* 2 (W) as described in experiment 1. While some reduction occurred in the numbers of leukocyte about the sixth day, there was no definite leukopenia, and the monkey apparently remained well.

Transfer of virus from the monkey to normal Aëdes.—Different groups, composed of twenty-four, twenty-eight, twenty-six, twenty-seven, twenty-eight, and thirty normal *Aëdes,* fed on the monkey *M. fuscatus* 4 (Y) November 8, 9, 10, 11, 12, and 13 and were designated lots 111–A, B, C, D, E, and F, respectively. November 27 these different lots, twenty-three of A, twenty-nine of B, eight of C, twenty-one of D, twenty-four of E, and twenty-three of F were combined and designated *A. ægypti* lot 111.

Test of mosquitoes for infectivity.—November 28, the following day, one hundred three mosquitoes of this lot (No. 111) fed on a susceptible volunteer (A.V. 60–S), who after an incubation period of six days developed typical dengue fever.

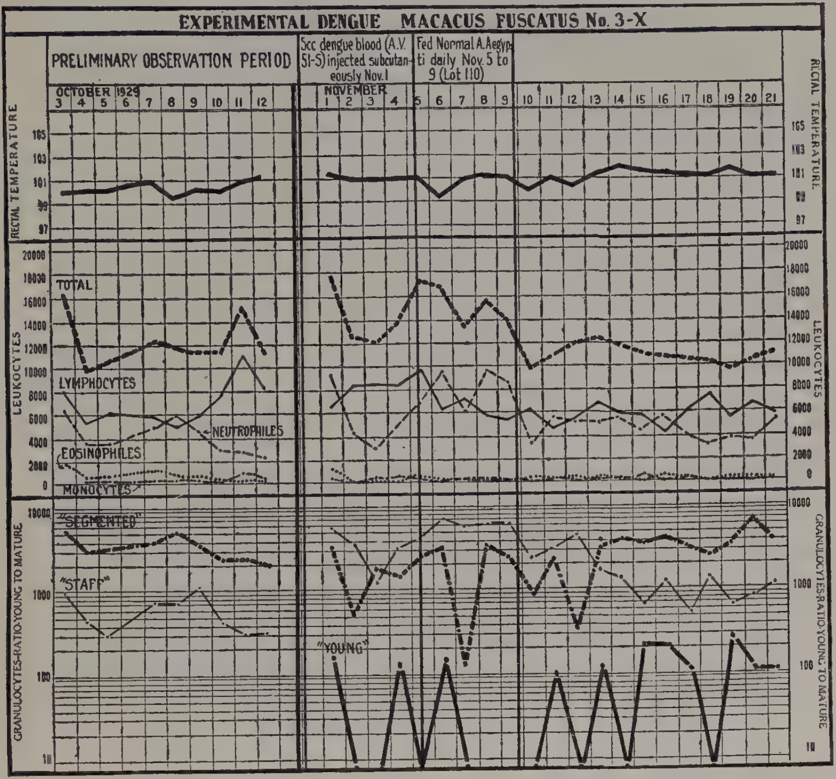


Fig. 55. Experimental dengue in *Macacus fuscatus* 3–X.

Comment.—In this experiment dengue was transmitted to a Japanese monkey (*M. fuscatus* 4 (Y), by *A. ægypti* lots 82 to 89 previously fed on *M. fuscatus* 2 (W), and from the monkey the infection was transmitted through *A. ægypti* lot 111 to man.

Experiment 4.—December 12, 1929. *M. fuscatus* 5 (Z), which had been protected from mosquitoes in screened cages since its departure from Tokyo, Japan, was bitten by eighty-four *A. ægypti* lot 110, which at least thirty-three days earlier had taken blood from *M. fuscatus* 3 (X). The monkey 5 (Z) showed no physical signs of dengue. The temperature and leukocyte counts were not significant.

Attempt to infect normal Aëdes.—Different groups of normal *A. ægypti* were fed on the fourth, fifth, and sixth days, thirty fed December 16, thirty fed December 17, and twenty-six fed December 18.

Test of mosquitoes for infectivity.—These mosquitoes were combined, designated lot 136, and given an opportunity to feed on a susceptible human volunteer (A.V. 69–K) at 9.30 a. m., January 13, 1930. Seventy mosquitoes of lot 136 took blood, but failed to produce dengue in this volunteer.

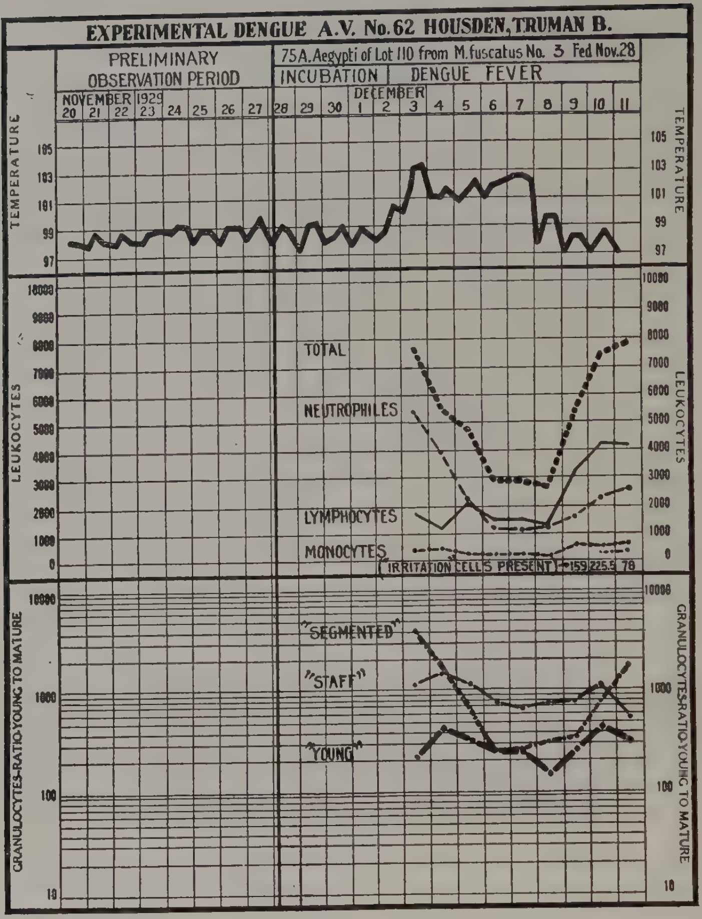


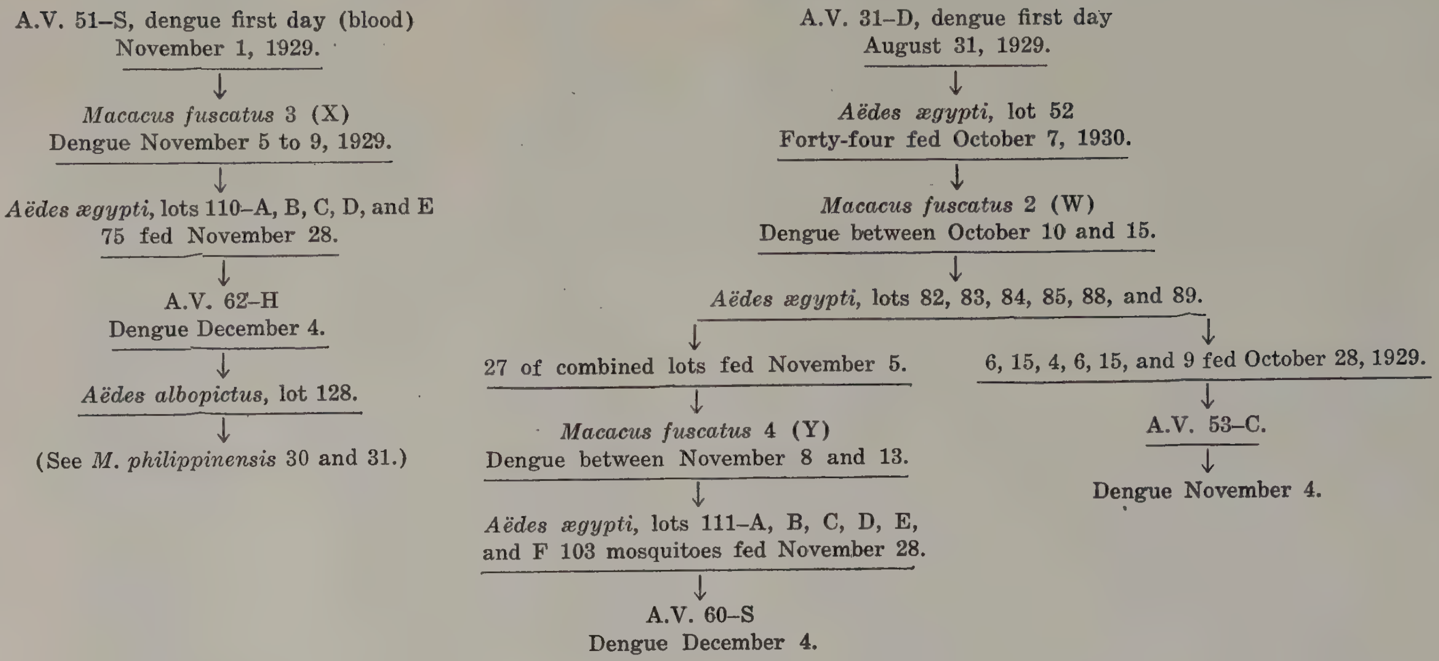
FIG. 56. Experimental dengue in American volunteer 62.

Comment.—While it is possible that this monkey, No. 5 (Z), was immune to dengue, the fact that it was tested for the presence of virus only on the fourth, fifth, and sixth days after the infectious feeding might account for the failure to prove that infection occurred.

SUMMARY

Dengue was produced experimentally in three of four Japanese monkeys (*M. fuscatus*). No significant diagnostic reactions were observed, but it was proved that the monkeys were infected by transfer of the virus through *Aëdes ægypti* to human volunteers and to monkeys.

CHART 4.—*Transfer of dengue virus through Japanese monkeys.*


A.V. 51–S, dengue first day (blood)
November 1, 1929.
↓
Macacus fuscatus 3 (X)
Dengue November 5 to 9, 1929.
↓
Aëdes ægypti, lots 110–A, B, C, D, and E
75 fed November 28.
↓
A.V. 62–H
Dengue December 4.
↓
Aëdes albopictus, lot 128.
↓
(See M. philippinensis 30 and 31.)
A.V. 31–D, dengue first day
August 31, 1929.
↓
Aëdes ægypti, lot 52
Forty-four fed October 7, 1930.
↓
Macacus fuscatus 2 (W)
Dengue between October 10 and 15.
↓
Aëdes ægypti, lots 82, 83, 84, 85, 88, and 89.
↓
27 of combined lots fed November 5.
↓
Macacus fuscatus 4 (Y)
Dengue between November 8 and 13.
↓
Aëdes ægypti, lots 111–A, B, C, D, E,
and F 103 mosquitoes fed November 28.
↓
A.V. 60–S
Dengue December 4.
↓
6, 15, 4, 6, 15, and 9 fed October 28, 1929.
↓
A.V. 53–C.
↓
Dengue November 4.

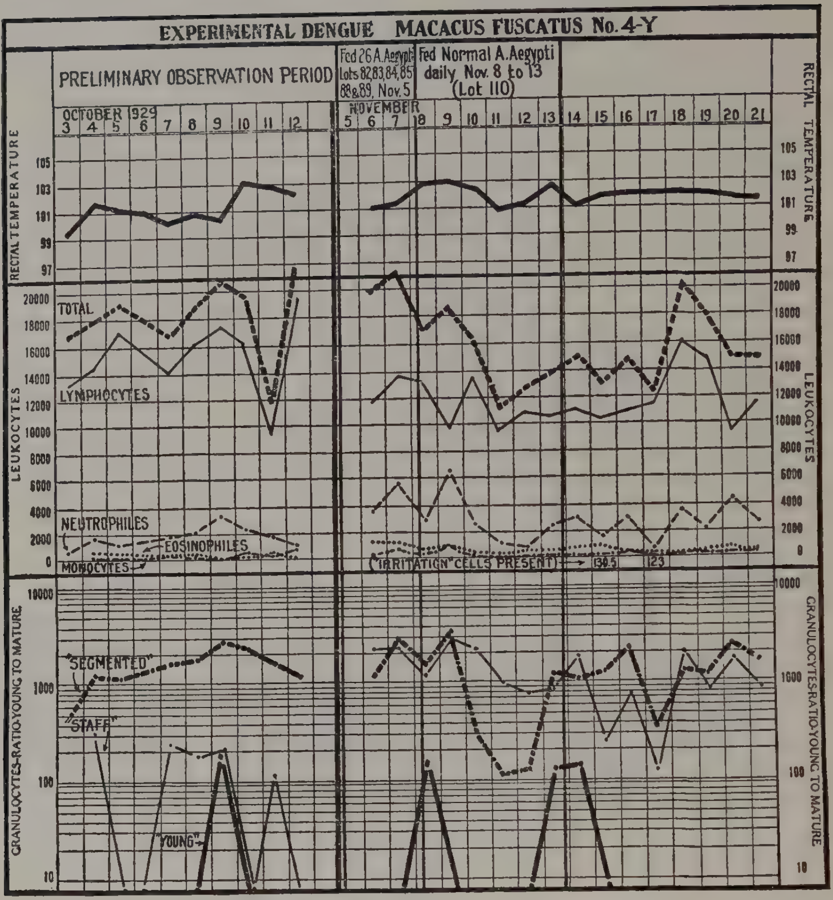


FIG. 57. Experimental dengue in *Macacus fuscatus* 4-Y.

MACACUS PHILIPPINENSIS

Seven monkeys (*Macacus philippinensis*) Nos. 22, 23, 25, 26, 27, 28, and 29, which had been kept unscreened for several years in the animal house of the Bureau of Science in Manila, were used in the following experiments:

Infective inoculations.—August 2, 1929, a suspension of twenty-five infective *A. ægypti* of lot 30, and twenty-five *A. ægypti* of lot 32 were macerated in a mortar with sterile sand, suspended in 14 cubic centimeters of physiologic salt solution, filtered through a wisp of cotton, and injected into the different monkeys as follows: *M. philippinensis* Nos. 22, 23, 27, 28, and 29 each received 1 cubic centimeter of mosquito suspension intracardially and 0.4 cubic centimeter subcutaneously; monkey 26 received 3

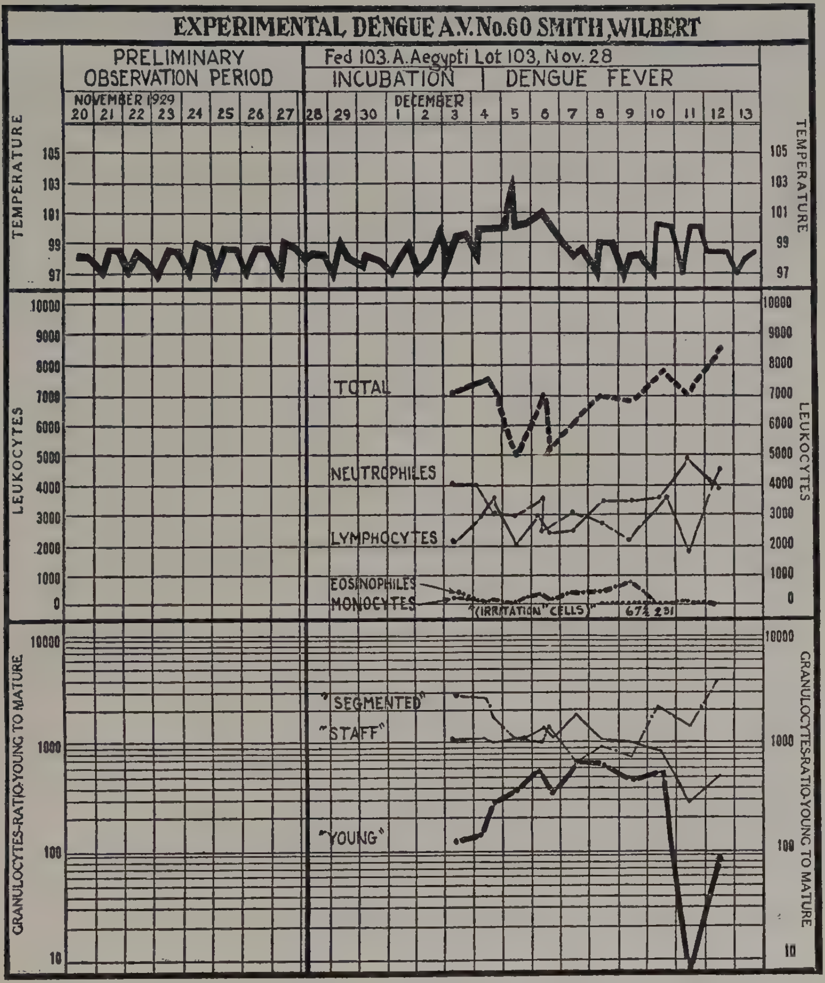


Fig. 58. Experimental dengue in American volunteer 60.

cubic centimeters intracardially and 1.5 cubic centimeters subcutaneously; while monkey 25 received 1 cubic centimeter subcutaneously.

Attempt to transfer virus from monkeys to normal Aëdes.—After these injections each monkey was exposed daily, August 5, 6, 7, 8, and 9, to different lots of mosquitoes, each composed of twenty normal *A. ægypti*. The numbers of mosquitoes which took blood each day are shown in Table 37.

As indicated in Table 37, the five groups of mosquitoes fed on each monkey were then combined in a single cage and given lot numbers as follows: The mosquitoes from monkey 22 were called lot 92–A; from monkey 23, lot 92–B; from monkey 25, lot 92–C; from monkey 26, lot

92–D; from monkey 27, lot 92–E; from monkey 28, lot 92–F; and from monkey 29, lot 92–G.

TABLE 37.—*Numbers of mosquitoes that fed on Macacus monkeys.*

Macacus philippinensis No.	Number of macerated dengue-infected *Aëdes* infected August 2, 1929.		Evidence of reaction.	Number of normal *A. ægypti* fed on monkeys August 5 to 9.					
	Intracardially.	Subcutaneously.		5	6	7	8	9	Total.
22	3.6 (1 cc)	1.4 (0.4 cc)	None	20	15	11	11	11	68 (lot 92–a).
23	3.6 (1 cc)	1.4 (0.4 cc)	do	20	16	13	9	9	67 (lot 92–b).
25		3.6 (1.0 cc)	do	16	20	14	13	11	74 (lot 92–c).
26	10.7 (3 cc)	5.4 (1.5 cc)	do	21	17	16	9	13	76 (lot 92–d).
27	3.6 (1 cc)	1.4 (0.4 cc)	do	19	17	7	15	14	72 (lot 92–e).
28	3.6 (1 cc)	1.4 (0.4 cc)	do	20	19	14	12	10	75 (lot 92–f).
29	3.6 (1 cc)	1.4 (0.4 cc)	do	14	18	14	19	13	78 (lot 92–g).

Macacus philippinensis No.	Test for infectivity of mosquitoes.			
	Number fed.	American volunteer.	Date.	Result.
22	9	46–L	September 23	Negative.
23	14	43–D	September 21	Do.
25	28	42–H	September 21	Dengue fever September 26.
26	33	41–F	September 16	Negative.
27	15	44–F	September 21	Do.
28	5	40–W	September 14	? (Immune).
29	28	45–V	September 21	Negative.

TESTS TO DETERMINE INFECTIVITY OF MOSQUITOES. LOTS 92–A, B, C, D, E, F, AND G

Experiment 5.—In order to determine whether it was worth while to test the different mosquito lots from the seven monkeys separately, lots 92–A, B, C, D, E, F, and G were first tested for infectivity as a group by giving them all an opportunity to feed on a susceptible volunteer (A.V. 35–E), who had been observed in a screened cubicle for eight days previous to the experiment. This volunteer was bitten by an unknown number of mosquitoes from each of the respective lots, and also by *A. ægypti* of lot 93, which were later proved to be free from dengue. After an incubation period of seven days the man developed a typical attack of dengue. Comment: The occurrence of dengue in A.V. 35–E indicated that one or more of the monkeys (22, 23, 25, 26, 27, 28, or 29) had been infected with dengue, and that the infection had been transmitted to man by some of the mosquitoes tested.

Experiment 6.—In order to confirm the results obtained in test "a," seventeen, twenty-four, thirty-seven, thirty-six, twenty-nine, twenty-three,

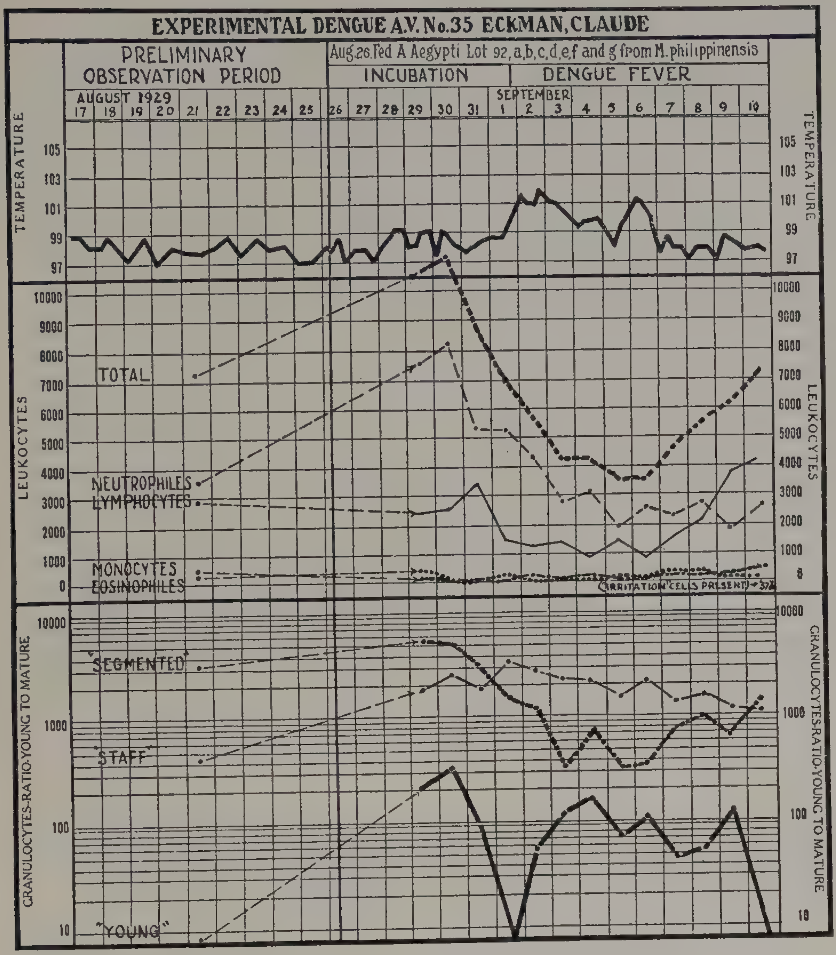


Fig. 59. Experimental dengue in American volunteer 35.

and thirty-seven *A. ægypti* of the respective lots, were again tested for infectivity September 5 by feeding them on another susceptible volunteer (A.V. 38–H). After an incubation period of five days this volunteer also developed typical dengue fever. Comment: This second positive test confirmed the first and proved that one or more of the monkeys had been infected with dengue.

In order to determine the number of monkeys infected, the following tests were made in which each of the seven lots of *A. ægypti* (92–A, B, C, D, E, F, and G) were fed separately on a different human volunteer.

Experiment 7.—*Aëdes ægypti* of lot 92–A from *M. philippinensis* 22. September 23, 1929, nine *A. ægypti* which remained alive of the sixty-eight of lot 92–A which at least forty-five days earlier had fed on monkey 22, took blood this date from a susceptible volunteer (A.V. 46–L). As dengue

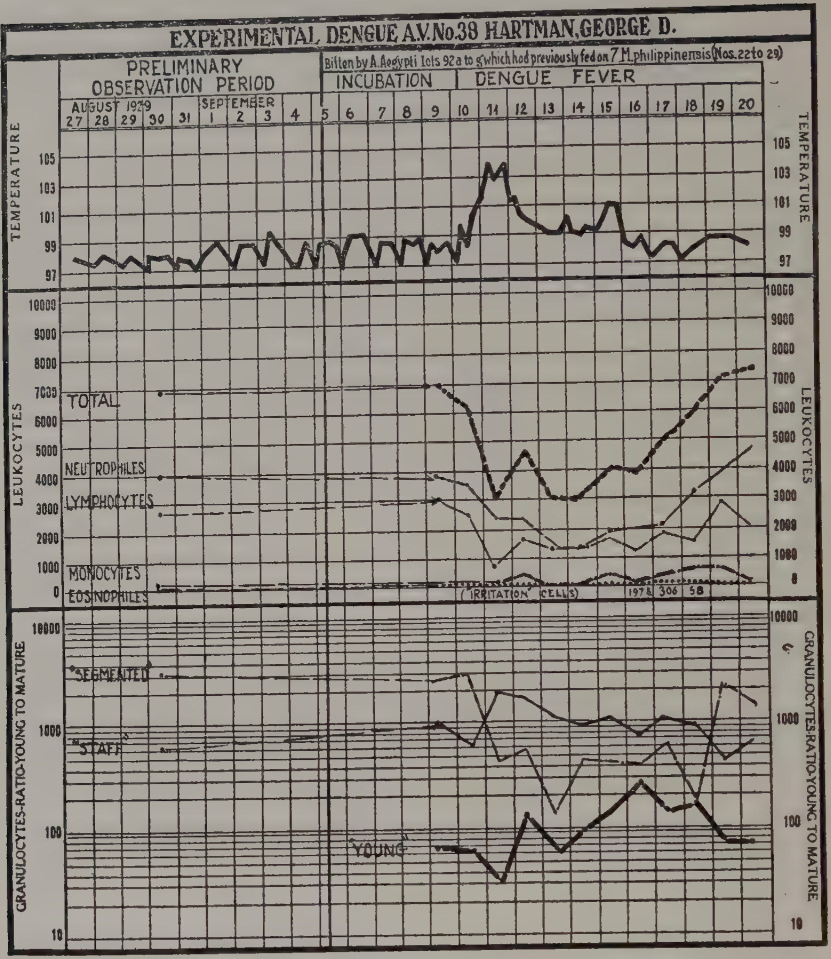


FIG. 60. Experimental dengue in American volunteer 38.

did not result from this feeding, it is apparent that the remaining mosquitoes of lot 92–A were not infected. While lot 92–A may have originally contained infected mosquitoes that had died before this test, there is no proof that this occurred. There was no indication that *M. philippinensis* 22 had been infected with dengue.

Experiment 8.—Aëdes ægypti, lot 92–B, from *M. philippinensis* 23. September 21, 1929, fourteen *A. ægypti* which remained alive of the sixty-seven of lot 92–B which had fed at least forty-three days earlier on monkey 23, took blood this date from a susceptible volunteer (A.V. 43–D). Since this man failed to develop dengue during the usual incubation period, it is apparent that the mosquitoes used of lot 92–B had not been infected by feeding on *M. philippinensis* 23. There was no evidence to prove that monkey 23 had been infected with dengue.

Experiment 9.—Aëdes ægypti, lot 92–C, from *M. philippinensis* 25. September 21, 1929. Twenty-eight of the *A. ægypti* of lot 92–C, which remained of the seventy-four that had fed at least forty-three days earlier on monkey 25, took blood this date from a susceptible volunteer (A.V. 42–H). That some of these mosquitoes were infected was proved by the fact that after an incubation period of five days this volunteer developed typical dengue fever.

Experiment 10.—Aëdes ægypti, lot 92–D, from *M. philippinensis* 26. September 16, 1929 (noon). Thirty-three *A. ægypti* of lot 92–D, which remained of the seventy-five that had fed thirty-eight or more days earlier on monkey 26, fed this date on a susceptible volunteer (A.V. 41–F). Dengue did not result.

Experiment 11.—Aëdes ægypti, lot 92–E, from *M. philippinensis* 27. September 21, 1929, 9 a. m. Fifteen of the original seventy-two *A. ægypti* of lot 92–E, which had taken blood forty-three or more days earlier from monkey 27, fed this date on a susceptible volunteer (A.V. 44–F). Dengue did not develop from this feeding.

Experiment 12.—Aëdes ægypti, lot 92–F, from *M. philippinensis* 28. September 14, 1929. Five of the original seventy-five *A. ægypti* of lot 92–F, which had taken blood thirty-six days earlier on monkey 28, fed this date on a volunteer (A.V. 40–W). Dengue did not result from this feeding, but since this volunteer was later shown to be immune the infectivity of lot 92–F mosquitoes remained in doubt.

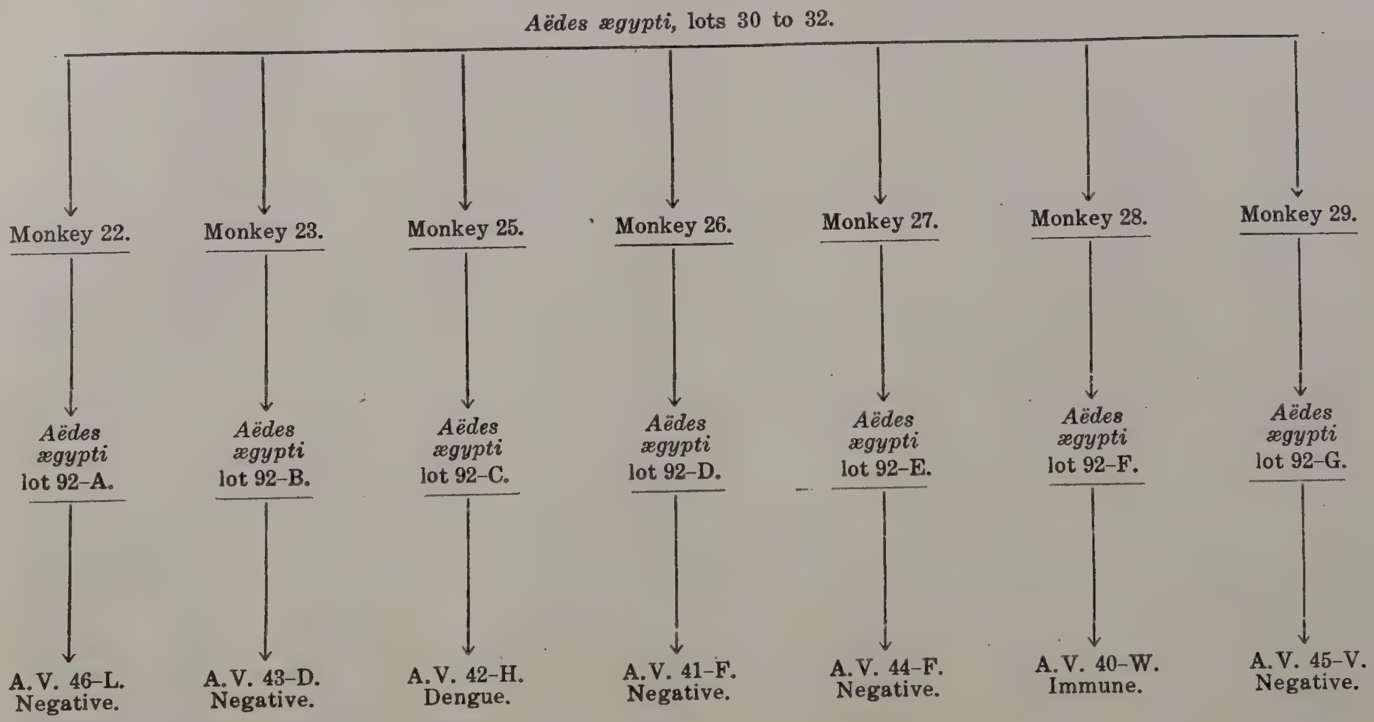
Experiment 13.—Aëdes ægypti, lot 92–G, from monkey 29. September 21, 1929, 9 a. m. Twenty-eight of the original seventy-eight *A. ægypti* of lot 92–G, which had taken blood at least forty-three days previously from monkey 29, were fed this date on a susceptible volunteer (A.V. 45–V). Dengue did not develop from this feeding. There was no proof that monkey 29 had dengue.

As it was suspected that a large proportion of *M. philippinensis* raised in localities where dengue is not endemic might be susceptible to the infection, monkeys were obtained from Mountain Province in northern Luzon in which region dengue does not occur. Three monkeys were caught at an elevation of about 4,000 feet, near Sagada, Philippine Islands, and were transported to Manila in boxes screened with 20-mesh wire netting. After periods of normal observation in mosquito-proof cages these three monkeys were used in the following dengue transmission experiments:

Experiment 14.—January 16, 1930, *M. philippinensis* 30 (Sagada A) was bitten by thirty-two infected *A. albopictus* of lot 128, which forty-three days previously had taken blood from an experimental case (A.V. 62–H) during the first day of dengue. The monkey failed to show any recognizable clinical evidence of infection.

However, normal *A. ægypti* were fed on the monkey, as follows: Twenty-five January 20, twenty-nine January 21, thirty January 22, twenty-five January 23, and twenty-seven January 24.

CHART 5.—*Diagrammatic chart indicating attempts to pass dengue virus through Macacus philippinensis monkeys obtained from an endemic dengue area of the Philippines.*


Aëdes ægypti, lots 30 to 32.
Monkey 22.
Monkey 23.
Monkey 25.
Monkey 26.
Monkey 27.
Monkey 28.
Monkey 29.
Aëdes ægypti lot 92-A.
Aëdes ægypti lot 92-B.
Aëdes ægypti lot 92-C.
Aëdes ægypti lot 92-D.
Aëdes ægypti lot 92-E.
Aëdes ægypti lot 92-F.
Aëdes ægypti lot 92-G.
A.V. 46-L. Negative.
A.V. 43-D. Negative.
A.V. 42-H. Dengue.
A.V. 41-F. Negative.
A.V. 44-F. Negative.
A.V. 40-W. Immune.
A.V. 45-V. Negative.

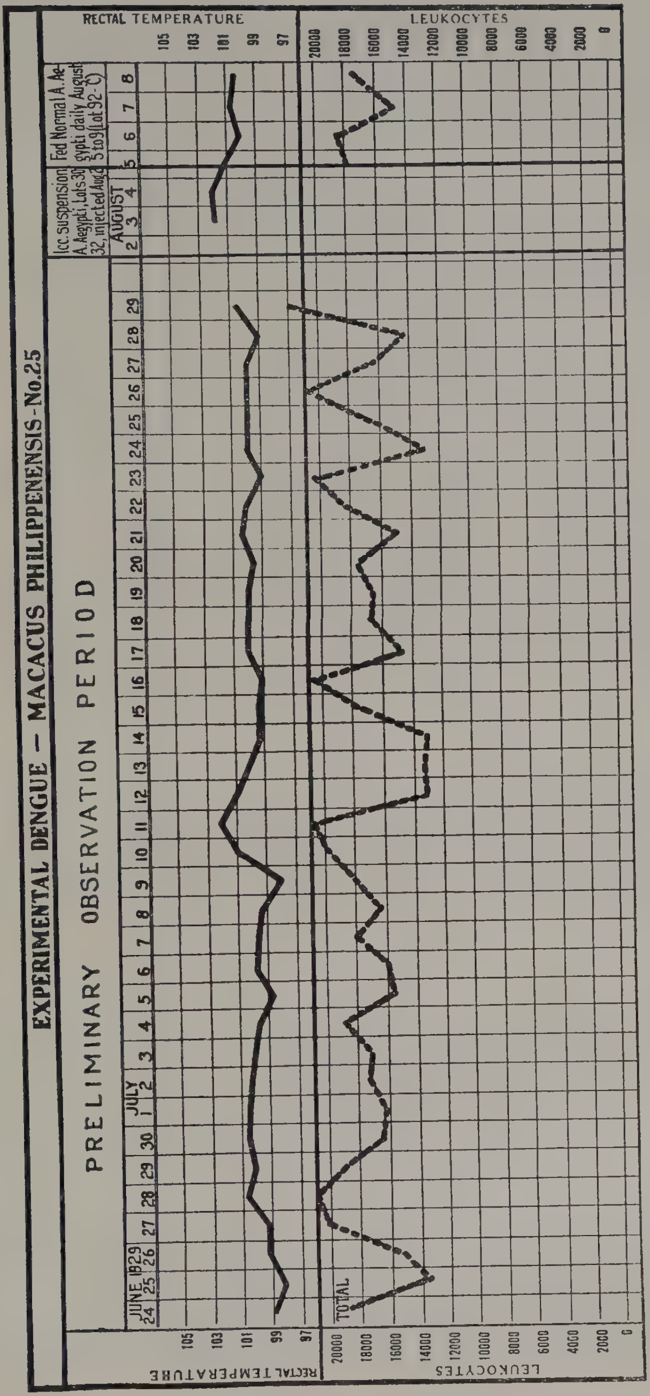


Fig. 61. Experimental dengue in *Macacus philippinensis* 25.

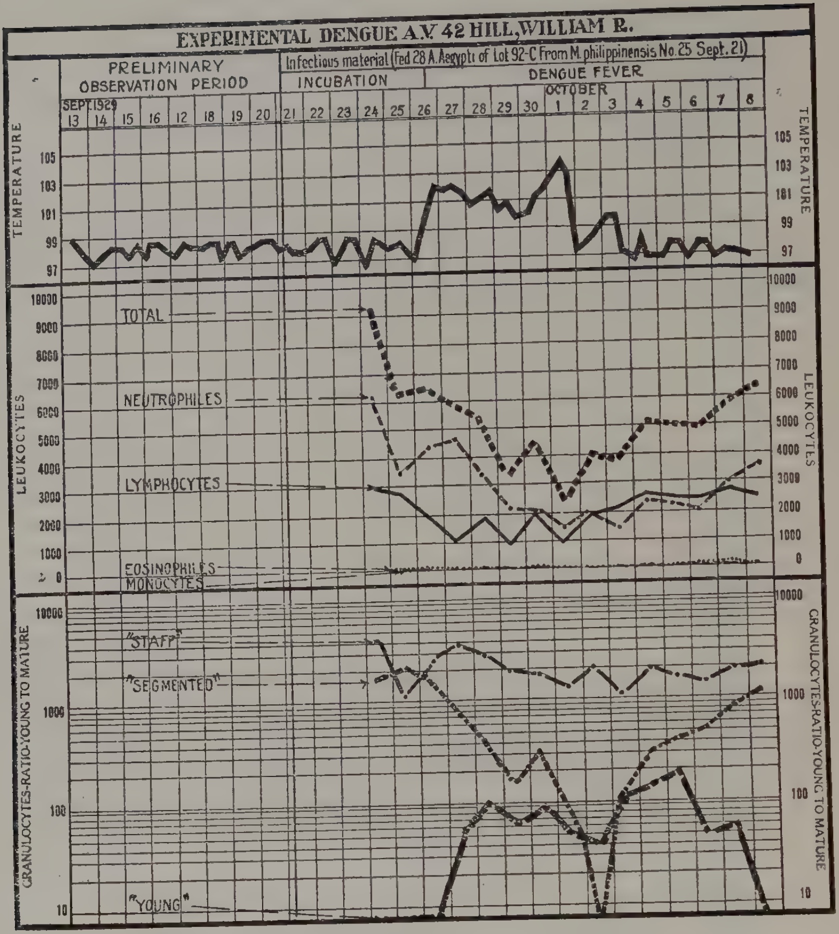


FIG. 62. Experimental dengue in American volunteer 42.

February 15, 1930 (10.30 a. m.) seventy-three of these five groups of mosquitoes, which had been combined and designated lot 156, were fed on a susceptible volunteer (A.V. 80–L). After an incubation period of six days the volunteer developed typical dengue fever.

February 24, 1930, an attempt was made to transmit dengue from monkey to monkey by feeding thirty-one *A. ægypti* of lot 156 on *M. philippinensis* 32 (Sagada C), but dengue was not proved in the latter monkey as is described in experiment 16.

Experiment 15.—January 16, 1930, *M. philippinensis* 31 (Sagada B) was bitten by thirty-five infected *A. albopictus* of lot 128, which forty-three days previously had taken blood from an experimental case of dengue (A.V. 62–H), during the first day of the disease. The monkey failed to show any recognizable clinical evidence of infection. Normal *A. ægypti* were fed on the monkey as follows: Twenty-seven January 20, thirty

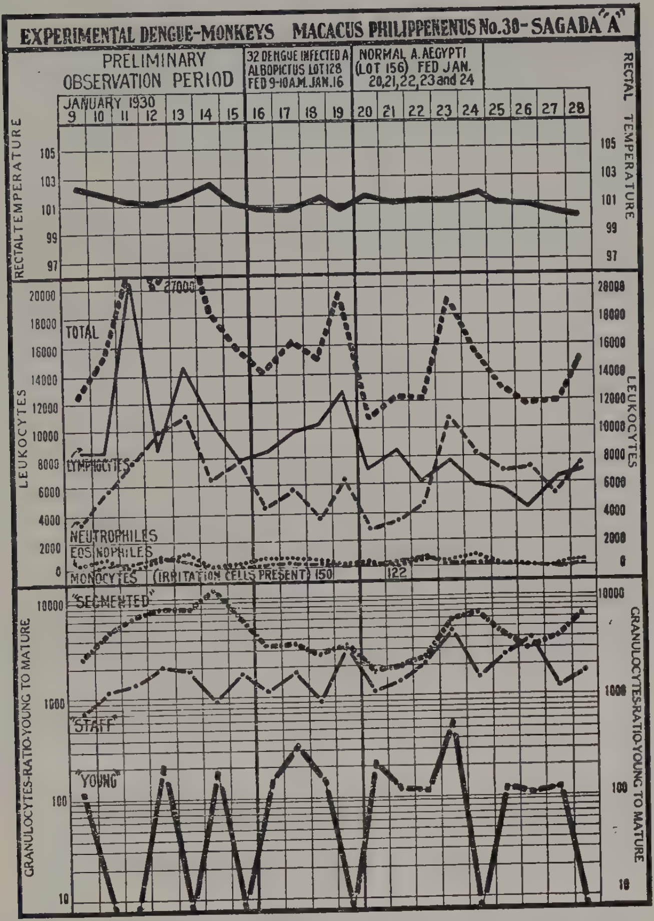


FIG. 63. Experimental dengue monkeys *Macacus philippinensis* 30–Sagada "A."

January 21, twenty-six January 22, twenty-nine January 23 and thirty-one January 24. February 15 eighty-seven mosquitoes of these combined groups, which had been designated lot 157, were tested for infectivity by feeding on a susceptible volunteer (A.V. 81–G). After an incubation period of five days this volunteer developed typical dengue fever.

Experiment 16.—February 24, 1930, *M. philippinensis* 32 (Sagada C) was bitten by thirty-one of the one hundred thirty-six *A. ægypti* remaining in lot 156, which had at least thirty-one days earlier fed on *M. philippinensis* 30 (Sagada A). The infectivity of some of the mosquitoes of lot 156 had been proved earlier by their transmission of dengue to a human volunteer. The monkey (32–C) was tested for dengue by feeding on it groups of normal *A. ægypti* as follows: Twenty-seven February 28, thirty-three March 1, thirty-three March 2, twenty-six March 3, and twenty-

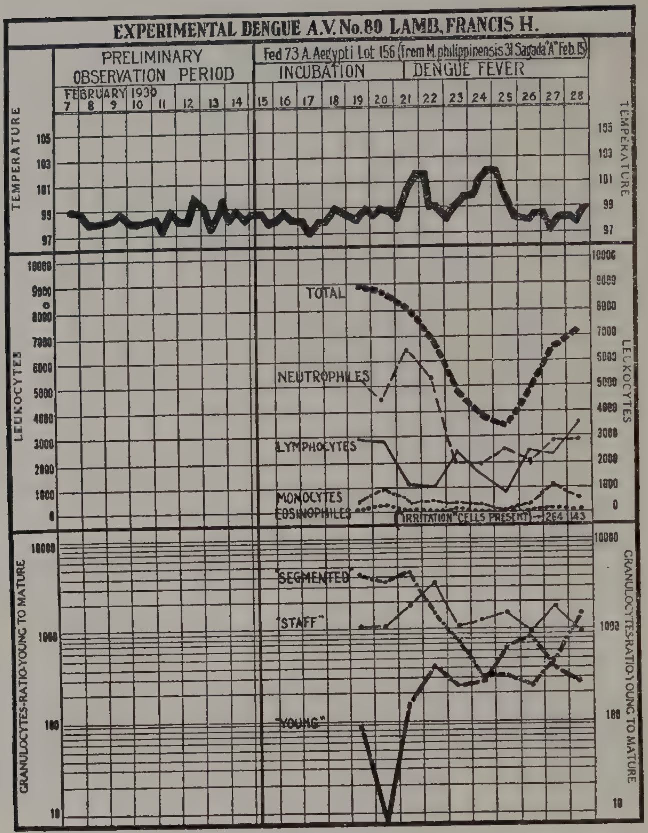


FIG. 64. Experimental dengue in American volunteer 80.

nine March 4. March 22 (11 a. m.) seventy-six of these combined groups of mosquitoes, called lot 172, fed on a susceptible volunteer (A.V. 90–A), who failed to develop dengue. Comment: There was no evidence to indicate the occurrence of dengue in *M. philippinensis* 32 (Sagada C). It is, of course, possible that the infected mosquitoes in lot 156 had all died before feeding on this monkey, but there is no evidence to prove that this occurred.

DISCUSSION

Studies of daily temperature reactions and leukocyte counts on more than forty-one monkeys, inoculated with dengue virus, indicate that neither of these tests was of assistance in the diagnosis of dengue in these animals. The rectal temperature of apparently normal monkeys was found to vary considerably from day to day, and as a rule the temperature curves for the periods after inoculation showed similar daily variations. The numbers of leukocytes in blood collected from monkeys be-

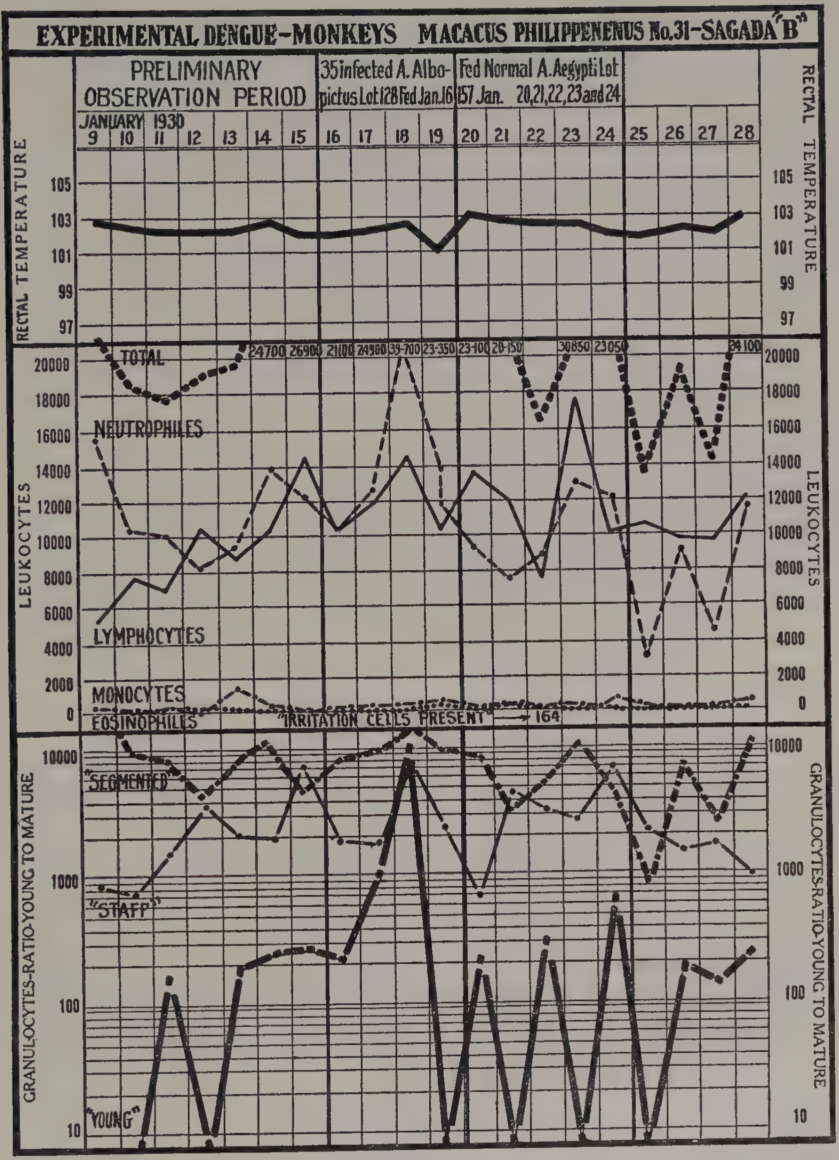
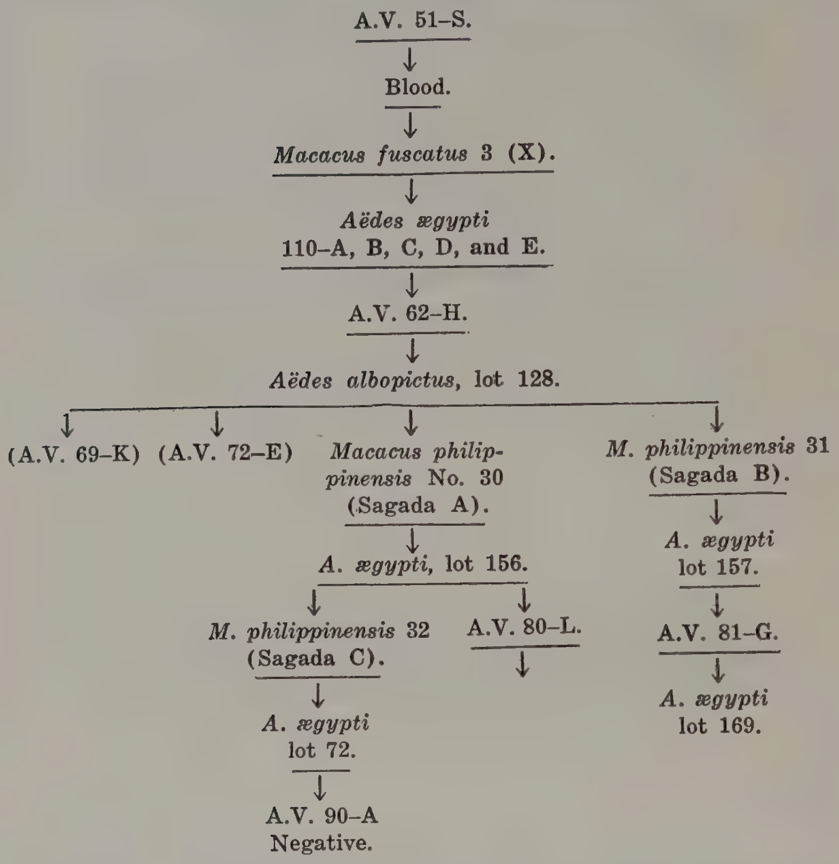


FIG. 65. Experimental dengue monkeys *Macacus philippinensis* 31–Sagada "B".

fore inoculation were also irregular, and the counts were often extremely high. Preliminary observations on normal monkeys showed that markedly different leukocyte counts were obtained in blood collected simultaneously from the ear, from the sole of the foot, and from the finger of a single animal, and for this

CHART 6.—*Indicating transfer of dengue virus to and from Philippine monkeys obtained from a dengue-free location.*


A.V. 51–S.
Blood.
Macacus fuscatus 3 (X).
Aëdes ægypti 110–A, B, C, D, and E.
A.V. 62–H.
Aëdes albopictus, lot 128.
(A.V. 69–K)
(A.V. 72–E)
Macacus philippinensis No. 30 (Sagada A).
M. philippinensis 31 (Sagada B).
A. ægypti, lot 156.
A. ægypti lot 157.
M. philippinensis 32 (Sagada C).
A.V. 80–L.
A.V. 81–G.
A. ægypti lot 72.
A. ægypti lot 169.
A.V. 90–A Negative.

reason most of the counts in these experiments were made in blood from a finger or toe. Whether due to technical difficulties or to variations in their leukocyte counts, very little evidence was obtained of a definite leukopenia, even in the animals known to have been infected with dengue. However, it may be significant that, as has been reported by previous workers, some of the leukocyte curves showed a suggestive drop after a period corresponding to the incubation period required for the development of dengue in man. The technic employed in proving that certain of the monkeys had developed dengue has certain limitations because of the possibility that in some instances a negative result might have been obtained because the mosquitoes fed during the particular time of the monkey's infectivity might

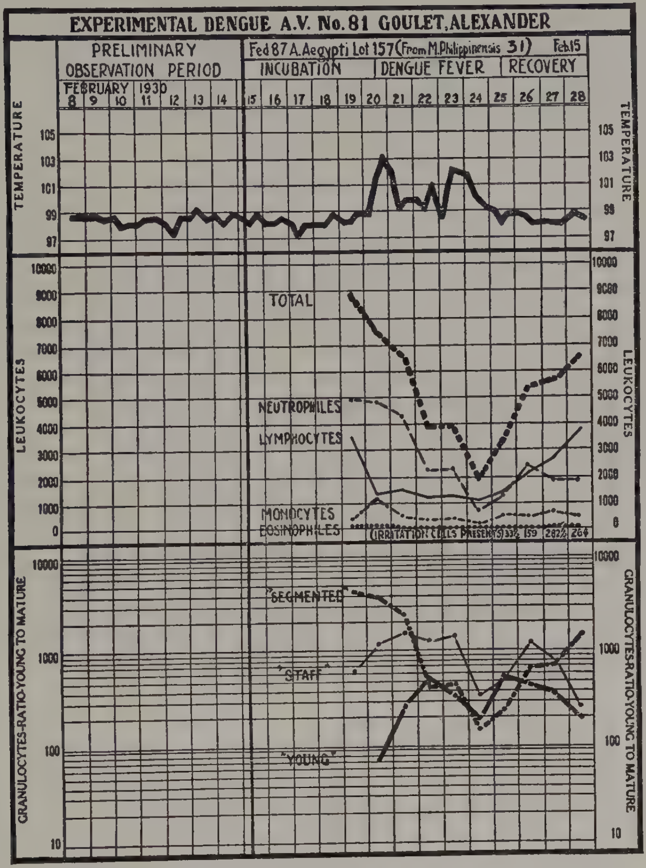


FIG. 66. Experimental dengue in American volunteer 81.

have died before the combined lots for the entire period were tested on volunteers. However, in spite of the fact that the method employed is too uncertain and tedious for routine diagnosis, it has served an important purpose in this study by furnishing proof that certain monkeys may be infected with dengue, and that the virus multiplies in their blood until it reaches concentrations sufficient to result in its transmission through *Aëdes ægypti* to other monkeys and to man. The significance of the one negative transmission experiment with *M. rhesus* must remain in doubt, since this animal was tested for infectivity only on the fifth day after the inoculation of virus. However, dengue infection was proved in three of the four monkeys from Japan (*M. fuscatus*), in one of seven monkeys (*M. philippinen-*

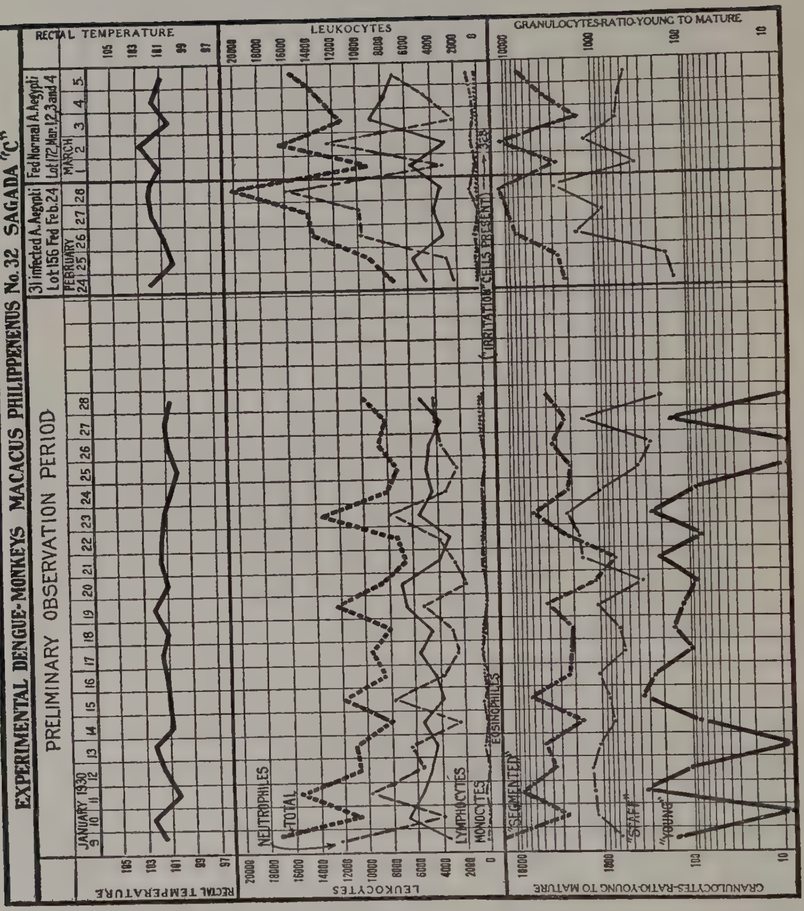


FIG. 67. Experimental dengue monkeys *Macacus philippinensis* 32–Sagada "C."

sis) obtained in or around Manila, where dengue is endemic, and in two of the three monkeys of the same species from a nonendemic area in the mountains of northern Luzon. Considering the limitations of the method used, it seems probable that monkeys other than those identified might also have been infected.

The knowledge that dengue can be transmitted by infected mosquitoes to monkeys and that from the latter the infection can be retransmitted through mosquitoes to other monkeys and to man is of considerable interest and importance because of its bearing on the epidemiology of dengue. It would appear that in certain localities the monkey may be an important factor in the spread of this desease.

The fact that susceptible individuals of a single species (*M. philippinensis*) were found both in endemic and nonendemic areas, but that a larger proportion of the former seemed resistant to infection, appears to indicate that immunity to dengue is probably acquired as a result of accidental infection at some time between birth and adult life. If this is true, it would not appear improbable for the virus of dengue to be maintained during long periods of time, either in the jungle or in sparsely populated localities by passage through the young of monkeys alone.

These observations are paralleled by the results obtained experimentally in attempts to produce dengue in natives from different sections of the Philippines. In an earlier chapter it is shown that adult natives living in endemic areas are generally immune to dengue, while others living in the nonendemic Mountain Province of Luzon, at elevations of 4,000 to 5,000 feet, were found to be susceptible. It is apparent that monkeys, particularly in the Philippines, may be an important factor in the spread of dengue.

SUMMARY AND CONCLUSIONS

1. Ten chickens, two lizards, six mice, twelve guinea pigs, seven rabbits, and twenty-eight monkeys (*Macacus philippinensis*) which were obtained in dengue-endemic areas, were inoculated with blood from dengue patients or other monkeys, emulsions of infective mosquitoes, or were bitten by infective mosquitoes.

2. Five Japanese monkeys, *Macacus fuscatus;* five Philippine monkeys, *M. philippinensis,* from non-dengue-endemic areas; five

monkeys, *M. rhesus,* obtained from the Zoölogical Park, Washington, D. C., and three guinea pigs, born and raised in a mosquito-proof cage, were either inoculated with infective blood or were bitten by dengue-infected mosquitoes.

3. Although observations were made of the temperature and leukocyte counts daily, before and after inoculations, no consistent or typical diagnostic sign of dengue was observed, even in the monkeys that were proved to be infected with dengue.

4. However, as has been observed by others, certain of the animals developed slight changes, either in temperature reactions or in the numbers of leukocytes, about the fourth, fifth, or sixth day after inoculation, and these were at least suggestive of a specific reaction.

5. Attempts were made to determine the presence or absence of infection in chickens, guinea pigs, and monkeys by feeding normal *Aëdes* mosquitoes on them on certain arbitrarily selected days after the inoculation, and later testing the mosquitoes on susceptible human volunteers.

6. Four cockerels obtained in Manila, which previous to their experimental use were probably exposed to dengue-infected mosquitoes, were not infected with dengue, as shown by the failure of mosquitoes, fed on these chickens on the third to eighth days inclusive, to transmit dengue to a susceptible volunteer.

7. Three guinea pigs, fifteen to eighteen days of age, which from birth had been protected from mosquitoes, were tested for dengue infection by means of mosquitoes that had fed on these animals in one instance during the fourth, fifth, sixth, seventh, and eighth days and on two other guinea pigs on the fifth, sixth, seventh, and eighth days following their potential infection. The mosquitoes failed to infect susceptible volunteers.

8. In the transmission experiments with monkeys the results were as follows:

(*a*) (Animals tested for infectivity on the fifth and sixth days only.) In one *M. rhesus* from Washington, one *M. fuscatus* from Tokyo, two *M. philippinensis* from Manila, Philippine Islands, and two *M. philippinensis* from Sagada, Philippine Islands, the infection was not proved by the transfer of virus to man.

(*b*) (Animals tested for infectivity on several days between the third and eighth.) Dengue infection was produced in three of four *M. fuscatus* from Tokyo, in one of seven *M. philippinensis* from Manila, and in two of three *M. philippinensis* from Sagada, a dengue-free locality.

9. The apparently greater prevalence of susceptible animals among monkeys from localities where dengue is not prevalent seems to indicate that, as with the adult native Filipino, immunity is probably acquired as a result of natural infections during early life.

10. These observations indicate that negative conclusions should not be drawn concerning the possibility of infecting lower animals with dengue unless (*a*) the animals tested are known to have been protected throughout their lives from the bites of infected mosquitoes, and (*b*) unless they are tested daily from the fourth to the eighth or the ninth day after the introduction of the virus by transfer of blood either directly or through the mosquito vectors to susceptible volunteers.

11. It seems probable that further experiments, conducted with these requirements in mind, might show that animals other than monkeys can be infected with dengue.

12. The proof offered here establishes the fact that dengue can be transmitted to monkeys of the species *M. fuscatus* and *M. philippinensis* by infected *Aëdes;* that the virus multiplies in the blood without producing recognizable typical changes in the temperature or the leukocyte counts; that at some time between the third and the ninth day after infection, the virus may be passed to other monkeys by blood inoculation or may be transferred through *Aëdes* to other monkeys or man; and that in one monkey a single attack produced an immunity which lasted for several days.

13. Since these observations indicate that monkeys are able to take part in the natural dissemination and maintenance of virus in endemic tropical localities, they are of considerable importance to any consideration of the epidemiology of dengue.

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At times it was difficult to obtain suitable men who were willing to volunteer for this work and in many instances they were secured only by the diligent personal efforts of the following medical officers: Lieut. Col. Henry C. Pillsbury, M. C., surgeon, Fort Stotsenburg; Maj. Bertram F. Duckwall, M. C., and Maj. James E. Poor, M. C., surgeons, 31st Infantry; Maj. John Dibble, M. C., attending surgeon, Fort William McKinley; Maj. William

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Capt. John H. Kintner, V. C., who reported for duty with the research board a few months before the completion of this study, gave his entire time to assist in the compilation of the data and in the preparation of the manuscript.

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The various phases of these investigations were planned and carried out by the authors working coöperatively and without any sharp division of duties. Expert technical assistance was rendered by the following members of the Medical Department, three of whom had been trained for their special duties during the investigations made by Siler, Hall, and Hitchens. Staff Sergeant Jesse F. Rhodes was the assistant in general charge of the technical work and directly responsible for the breeding and handling of mosquitoes. He was aided in the mosquito breeding by Private 1st class Bonifacio Reyes (PS) (laboratory specialist, 2d class), who had been engaged in similar work during the previous six years; and by Private 1st class Gervacio Sales (PS) (laboratory specialist, 3d class), who had also been on duty with the board during the previous four years. Sergeant Carl G. Heilmann and Private 1st class Hinton F. Miller assisted in many of the miscellaneous duties connected with the work, including the experiments on animals, the work in leukocyte counts, and the preparation of charts. The board's secretaries, Mrs. Nancy Lynham Carpenter and Miss Adele Scott, assisted in the preparation of protocols, records, and manuscript. Three other members of the enlisted personnel who were not actively engaged in the work on dengue, but who helped in various ways, were Staff Sergeant Thomas Reed; Private 1st class Joseph M. Hoff, M. D. (laboratory specialist, 1st class); and Private 1st class Gregorio D. Dumlao, (PS) (laboratory specialist, 3d class).

The more technical procedures were carried out in the laboratories of the board in the Bureau of Science. Fortunately, much of the apparatus and materials formerly used by Siler, Hall, and Hitchens, and later by Schule, were still available, including a laboratory-bred strain of *Aëdes ægypti* which were descendants of the original mosquitoes used by these previous workers. In general, the methods used for breeding mosquitoes and the types of cages used for breeding and feeding them on volunteers were similar to those described in the monograph published by the former investigators.

Soldiers of the United States Army and native Ifugaos were used in the production of experimental dengue in man. When possible American volunteers were selected from groups of soldiers who had recently arrived in the Philippines. Upon admission to the ward a complete physical examination was made, including routine examinations of urine and fæces, Wassermann tests, and daily temperature and leukocyte counts. All volunteers were protected from mosquitoes by keeping them in cubicles screened with 20-mesh copper wire within a screened ward of the Sternberg General Hospital, Manila, Philippine Islands. In practically all investigations concerned with transmission or identification of the virus the volunteers were first observed in the screened cubicles for eight or more days in order to make sure that they had not been infected previous to admission to the ward.

The diagnosis of dengue in each of the experimental volunteers was established by a careful study of the symptoms, including daily temperature and leukocyte counts, and, when indicated, by subsequent transfers of the virus through mosquitoes to other volunteers. Details of the different phases of the investigation are presented as separate articles or chapters and the records of the different lots of mosquitoes and volunteers will be given in the reprint of this article in Bureau of Science Monograph 29.

ILLUSTRATIONS

PLATE 1

Aëdes albopictus Skuse.

PLATE 2

FIG. 1. Apparatus for feeding mosquitoes through skin; *a*, lid of staining dish with guinea-pig skin clamped over shallow depression which is filled with mixture of normal blood and macerated infected mosquitoes; *b*, staining dish partially filled with water heated to 55° C.

2. *Aëdes ægypti* feeding through guinea-pig skin on a mixture of normal blood and macerated dengue-infected mosquitoes.

PLATE 3

Screened cubicles for isolation of human volunteers. (Photographed by United States Army Signal Corps.)

TEXT FIGURES

FIG. 1. Annual dengue admission rates per thousand white enlisted men in the United States Army, 1928.
2. Dengue compared with all other diseases contracted by American soldiers in the Philippine Islands, 1902–1928.
3. Distribution of dengue among Army posts in the Philippine Islands, 1922–1929.
4. Dengue fever at Fort Stotsenburg, Philippine Islands.
5. Dengue at Fort Mills, Corregidor, Philippine Islands.
6. Dengue fever among troops in Manila stationed at Fort William McKinley and Camp Nichols.
7. Seasonal occurrence of dengue fever at Army posts in the Philippine Islands.
8. Comparison of dengue incidence in white American and native Filipino troops in the Philippine Islands, 1915–1928.
9. Schematic representation of the passage of a single strain of dengue virus through various hosts.
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11. Experimental dengue in American volunteer 94.
12. Experimental dengue in American volunteer 39.
13. Experimental dengue in American volunteer 36.
14. Experimental dengue in American volunteer 57.
15. Experimental dengue in American volunteer 88.

16. Experimental dengue in American volunteer 47.
17. Experimental dengue in American volunteer 10.
18. Experimental dengue in American volunteer 29.
19. Experimental dengue in American volunteer 87.
20. Symptoms observed in experimental dengue.
21. Average leukocyte counts before, during, and after dengue fever.
22. Average leukocyte counts in cases of dengue with fever of two days duration.
23. Average leukocyte counts in cases of dengue with fever of three days duration.
24. Average leukocyte counts in cases of dengue with fever of four days duration.
25. Average leukocyte counts in cases of dengue with fever of five days duration.
26. Average leukocyte counts in cases of dengue with fever of six days duration.
27. Experimental dengue in Filipino volunteer 1.
28. Experimental dengue in Filipino volunteer 2.
29. Experimental dengue in Filipino volunteer 3.
30. Experimental dengue in Filipino volunteer 4.
31. Experimental dengue in Filipino volunteer 5.
32. Experimental dengue in Filipino volunteer 6.
33. Experimental dengue in Filipino volunteer 7.
34. Experimental dengue in Filipino volunteer 8.
35. Experimental dengue in Filipino volunteer 9.
36. Experimental dengue in Filipino volunteer 10.
37. Experimental dengue in Filipino volunteer 17.
38. Experimental dengue in Filipino volunteer 18.
39. Experimental dengue in Filipino volunteer 19.
40. Experimental dengue in Filipino volunteer 11.
41. Experimental dengue in Filipino volunteer 12.
42. Experimental dengue in Filipino volunteer 13.
43. Experimental dengue in Filipino volunteer 14.
44. Experimental dengue in Filipino volunteer 15.
45. Experimental dengue in Filipino volunteer 16.
46. Experimental dengue in Ifugao volunteer 1.
47. Experimental dengue in Ifugao volunteer 2.
48. Experimental dengue in Ifugao volunteer 3.
49. Experimental dengue in Ifugao volunteer 4.
50. Experimental dengue in guinea pig 14–A.
51. Experimental dengue in guinea pig 15–B.
52. Experimental dengue in guinea pig 16–C.
53. Experimental dengue in *Macacus fuscatus* 2–W.
54. Experimental dengue in American volunteer 53.
55. Experimental dengue in *Macacus fuscatus* 3–X.
56. Experimental dengue in American volunteer 62.
57. Experimental dengue in *Macacus fuscatus* 4–Y.
58. Experimental dengue in American volunteer 60.
59. Experimental dengue in American volunteer 35.

60. Experimental dengue in American volunteer 38.
61. Experimental dengue in *Macacus philippinensis* 25.
62. Experimental dengue in American volunteer 42.
63. Experimental dengue monkeys *Macacus philippinensis* 30–Sagada "A."
64. Experimental dengue in American volunteer 80.
65. Experimental dengue monkeys *Macacus philippinensis* 31–Sagada "B."
66. Experimental dengue in American volunteer 81.
67. Experimental dengue monkeys *Macacus philippinensis* 32–Sagada "C."

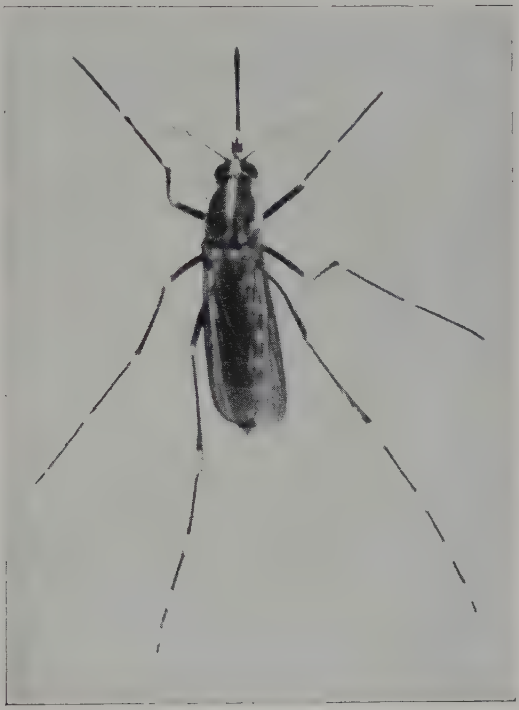
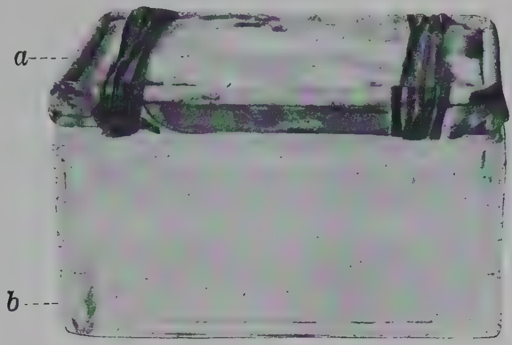


PLATE 1. AËDES ALBOPICTUS SKUSE.



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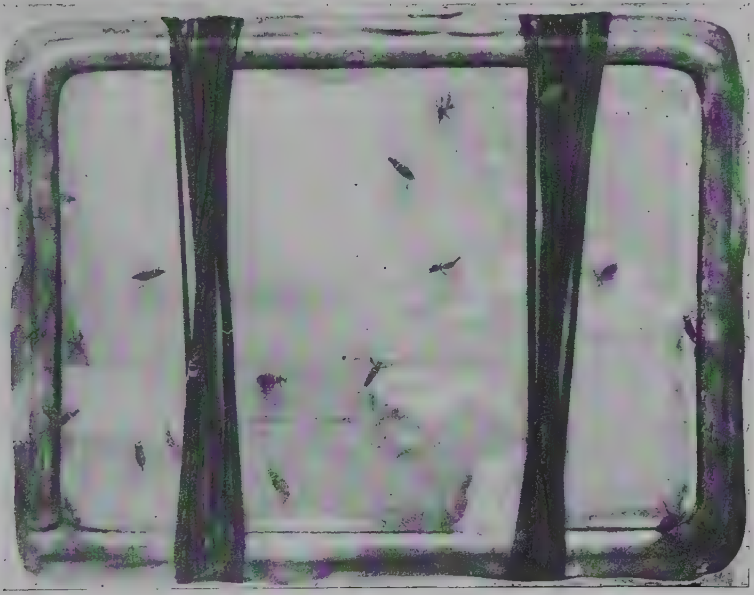

2

PLATE 2. APPARATUS FOR FEEDING MOSQUITOES THROUGH SKIN.

PLATE 2.

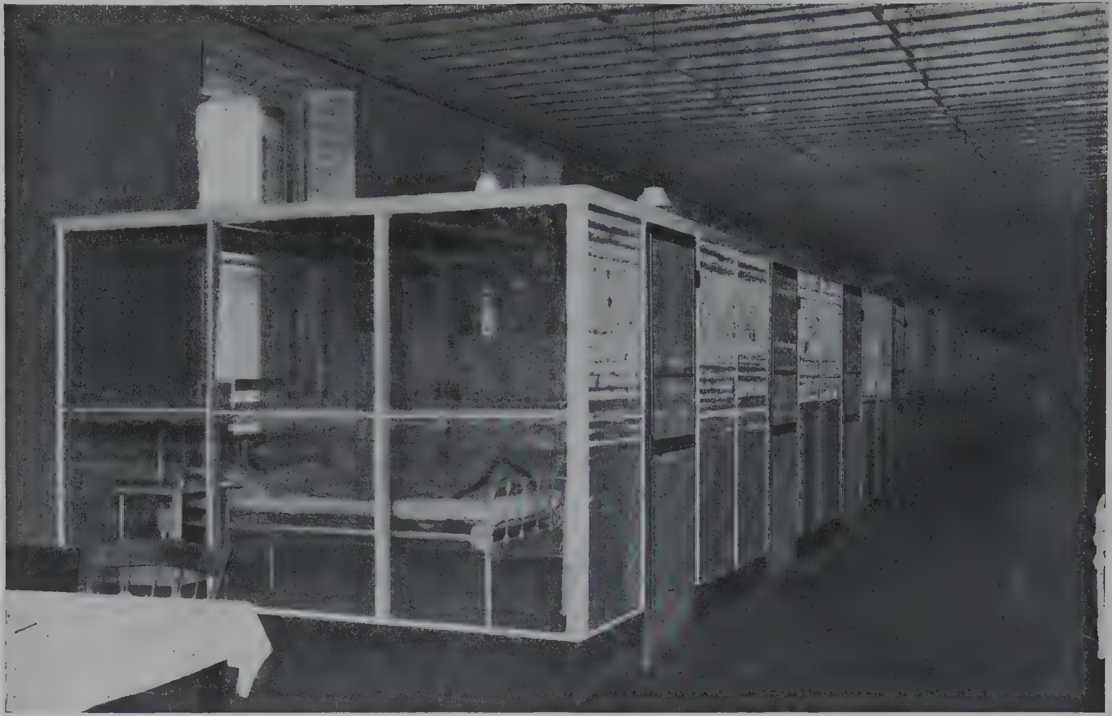

PLATE 3. SCREENED CUBICLES FOR ISOLATION OF HUMAN VOLUNTEERS.

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EIMERIA BUKIDNONENSIS, A NEW COCCIDIUM FROM CATTLE, AND OTHER COCCIDIAL PARASITES OF DOMESTICATED ANIMALS[1]

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TWO PLATES

The present report is the outcome of a survey conducted with the object of ascertaining the various forms of coccidial parasites found in domesticated animals. The survey was deemed necessary because, in spite of their recognized pathogenicity and common occurrence in domesticated animals in the Philippines, as reported by Schultz (1925), these organisms have not been studied systematically. Consequently, their identity, except that of one form found in a native cat and determined by Haughwout (1918) as *Isospora bigemina,* has long remained unknown. They have been merely referred to as "coccidia," as had been the practice elsewhere before the advent of modern protozoölogy.

The determination of these parasites was prompted by the following circumstances. For the last three years, usually during December and January, cases of bloody enteritis have been observed among young calves belonging to the dairy herd of the

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[1] The writer acknowledges with thanks his indebtedness to Mr. Felix B. Sarao and Drs. Victor Buencamino and Aniano Estores for kindly supplying him with coccidial oöcysts obtained from animals suffering from coccidiosis.

College of Agriculture, University of the Philippines, at Los Baños. Through the initiative of Mr. Felix B. Sarao, of the Department of Animal Husbandry, some of these cases were referred to the writer for diagnosis. A total of six animals were examined, and these were cow and carabao calves varying in age from one week to two months. The most important symptom presented was the frequent passing of liquid or semiliquid fæces containing rich admixtures of blood and mucus. The microscopic examination of the discharges revealed in every case the presence of coccidial oöcysts, which, as will be discussed below, represent two species of the protozoan genus *Eimeria*. The occurrence of the oöcysts in large numbers during the acute stages of the diarrhœa and their gradual disappearance at the onset of recovery (in the cases that recovered) show the etiological relationship of the parasites to the disease; which, in view of the principal symptom, is believed to be no other than the so-called red dysentery, or coccidiosis of cattle.

Recently, material has been obtained through the courtesy of Doctors Buencamino and Estores, of Manila, in the form of scrapings from the small intestine of a pup that died of a form of bloody enteritis. The case, according to Doctors Buencamino and Estores, was one of several pups belonging to the same litter that presented similar symptoms, which also ended fatally. At autopsy some of the animals showed intussusception in the region of the small intestine. The cases were correctly diagnosed as coccidiosis and, as will be shown below, the responsible parasite is a member of the genus *Isospora*.

ANIMALS AFFECTED AND SOURCE OF INFECTION

According to Smith and Graybill (1918) young calves (and this is probably true of pups and other young animals) become infected with coccidia soon after birth, but they may not show any manifestation of disease till several weeks later. This view brings up the questions: What is the source of the infection? How is it contracted? Regarding the first question, it has been shown that some adult animals, which may or may not have shown symptoms of coccidiosis during early life, may harbor coccidia without experiencing any apparent ill-effects from the presence of the parasites. Such animals act as carriers, and the coccidial oöcysts that they eliminate with their fæces constitute a source of danger to young susceptible animals. With reference to the manner of contracting the infection, it has also been shown that coccidial oöcysts are capable of retaining their viability for long periods even under adverse conditions. In view of this, it is most probable that young animals get the parasites by licking them up from the infected floors of their quarters or from the contaminated teats of the mammary glands of their mothers during the process of sucking.

Under certain conditions, adult animals that harbor coccidia and are, as already stated, apparently unmolested by the presence of the parasites, may also come down with acute symptoms of coccidiosis, as the following observations on two adult cats will show. These were animals that had been infected experimentally with surra organisms (*Trypanosoma evansi*) in connection with some other work in which the writer was engaged. They had previously been examined for coccidia and found to be infected with a species of the genus *Isospora*. The parasites were scarce, and the cats were apparently in the best of health. Soon after the appearance of the trypanosomes in the peripheral circulation, however, there was observed a sudden change in the character of the fæces of both animals. The evacuations became diarrhœic and bloody, and on examination they showed the presence of *Isospora* oöcysts in superabundance.

These are clearly cases of coccidiosis that were provoked by a lowering of the resistance due to secondary infections with surra. How often such provocations are brought about by other infectious diseases has not been determined, but it is now interesting to recall the observations of Schultz (1915, 1916) with reference to the common occurrence of coccidial organisms in the discharges of cattle and carabaos affected with rinderpest. This writer deserves much credit for his observations, but unfortunately he was tempted to ascribe the disease to the presence of these parasites.

METHODS

Collection of parasites.—The procedure adopted in the detection and collection of the parasites consisted mainly in emulsifying samples of fæces of individual animals in sterilized tap water, straining them through fine-meshed wire screens, and then collecting and examining the sediments after allowing the suspensions to stand in wide-mouthed cylinders for several hours or overnight. If a sample was found positive, it was diluted with a 2 per cent solution of potassium dichromate in order to allow the oöcysts to complete their development. In certain cases, in which the oöcysts were rare or from which large numbers of them were desired for critical study, a concentration method patterned after the direct centrifugal flotation (D. C. F.) method of Lane (1928) was used. The fæces were screened as usual, after the sediment had settled in a separatory funnel, the water was aspirated and replaced with concentrated sodium chloride solution. The mixture was shaken, and allowed to stand for at least thirty minutes in order to allow the oöcysts to float to the surface. The greater part of the salt solution was then removed, by opening the stopcock of the funnel, and was replaced with distilled water. The oöcysts were subsequently concentrated by centrifuging the contents of the funnel. The removal of the organisms from the concentrated salt solution is necessary because the high osmotic pressure of the latter causes the walls of the former to collapse sooner or later.

Measurements.—The measurements of the parasites were made with the use of a very carefully calibrated ocular micrometer. From twenty-five to forty oöcysts of each form of coccidium that was encountered in the survey were measured. In addition to recording the length and the breadth and giving the range in size of each species, it was thought advisable to conform to the recent practice and to the presumably more accurate method of expressing the measurements of parasitic Protozoa in terms of the mean, the standard deviation, and the coefficient of variation. These biometric constants, together with their probable errors, were calculated according to the following statistical formulæ:

Mean (M) $= \frac{\Sigma y}{n}$, in which y represents measurements (lengths or breadths) and n the number of oöcysts measured.

Standard deviation (S. D.) $= \sqrt{\frac{\Sigma y^2}{n} - M^2}$;

Coefficient of variation (C. V.) $= \frac{100 \times \text{S. D.}}{M}$;

Probable error of M $= \frac{\pm 0.6745 \times \text{S. D.}}{\sqrt{n}}$;

Probable error of S. D. $= \frac{\pm 0.6745 \times \text{S. D.}}{\sqrt{2n}}$;

Probable error of C. V. when C. V. is less than 10 $= \frac{\pm 0.6745 \times \text{C. V.}}{\sqrt{2n}}$;

Probable error of C. V. when C. V. is more than 10 $= \frac{\pm 0.6745 \times \text{C. V.}}{\sqrt{2n}} \times \left[1 + 2\left(\frac{\text{C. V.}}{100}\right)^2\right]$.

RESULTS OF EXAMINATIONS

The following were examined during the survey: Horses, cattle, carabaos, sheep, goats, pigs, dogs, cats, and chickens. One or more kinds of coccidia were found in the fæces of these animals, except the horse. There is no authentic record of these parasites in the horse, although Selan and Vittorio (1924) claimed to have seen an organism, which they named *Eimeria utinensis*, in the lungs and gall bladder of a horse in Italy. Before this, Pachinger (1886) and Sampson (1917) had described coccidial oöcysts in horses, but their descriptions, like that of Selan and Vittorio, are so meager that, as Wenyon (1926) states, it is impossible to tell if they were actually dealing with this group of parasites.

COCCIDIA OF CATTLE AND CARABAOS

Until recently, only one species of coccidium, *Eimeria zürnii*, has been recognized as being present in cattle and carabaos. Smith and Graybill (1918), in their studies on coccidial dysentery in calves in the United States, indicated the possible existence of more than one variety of these organisms in cattle. Their observations were later confirmed by Yakimoff and Galouzo (1927), who retained the name *E. zürnii* for the parasite, the oöcysts of which are roundish, with an average diameter of 17.1 microns and without a residual body in the sporocysts (Smith and Graybill's smaller type). For the egg-shaped oöcysts that have an average size of 31.5 by 21.6 microns and possess a residual body inside each sporocyst (Smith and Graybill's large type) they proposed the name *Eimeria smithi*.

Very recently Becker and Frye (1929) reported another coccidium from cattle in the United States under the name *Eimeria ellipsoidalis*. It is said to possess the following characters in common with *E. smithi:* There is a residual body inside each sporocyst, and the ratio of the average length to the average width is 1.47. It differs, however, from *E. smithi* in the following points: "(1) It (the oöcyst) is colorless and more inconspicuous. (2) The oöcyst wall is thinner, but does not thin out at the micropyle end proportionately to that of *E. smithi*. (3) It is ellipsoidal in shape. (4) It is considerably smaller in size" (20.0 to 26.0 by 13.0 to 17.0 microns; average size, 23.4 by 15.9 microns).

In our survey twenty-eight cattle and eleven carabaos were examined; seventeen of the former and five of the latter were found infected. *Eimeria zürnii* was present in only three cattle and in one carabao; the rest of the infected animals harbored *E. smithi.* The former is thus apparently rarer than the latter in adult animals, and yet in four of the six calves with dysentery it was the only organism involved. In the other two cases, although it was associated with *E. smithi,* it was present in predominating numbers. In the fæces of one bull that had been shipped to Manila from Bukidnon, Mindanao, for slaughter, an apparently new species was encountered together with *E. smithi.*

EIMERIA ZÜRNII (Rivolta, 1878). Plate 1, figs. 1, 2, 3 and 4.

The oöcysts are from spherical to ovoidal, with an average shape index of 1.10. They measure 13.5 to 20.2 by 12.3 to 18.0 microns. The average size of twenty-five oöcysts is $\frac{17.7 \pm 0.188}{16.1 \pm 0.151}$ microns,[1] with a standard deviation of $\frac{1.393 \pm 0.133}{1.121 \pm 0.107}$ and a coefficient of variation of $\frac{7.857 \pm 0.749}{6.962 \pm 0.662}$. The thickness of the oöcyst wall is uniform throughout, being about 1.1 microns. A distinct micropyle is not evident. The sporocysts are lemon-shaped and measure 7.8 to 11.2 by 5.1 to 5.8 microns. The oöcysts and sporocysts are devoid of any residual protoplasm.

EIMERIA SMITHI Yakimoff and Galouzo, 1927. Plate 1, figs. 5 and 6.

The oöcysts of this species are oval, with an average shape index of 1.51. They are larger than those of *E. zürnii,* measuring 28.8 to 39.6 by 19.8 to 27.0 microns. The average size of twenty-five oöcysts is $\frac{35.4 \pm 0.509}{23.5 \pm 0.324}$ microns, with a standard deviation of $\frac{3.780 \pm 0.360}{2.405 \pm 0.229}$ and a coefficient of variation of $\frac{10.678 \pm 1.038}{10.234 \pm 0.995}$. The oöcyst wall averages about 1.3 microns in thickness, except at the micropyle end where it is much thinner. In rare cases the oöcyst wall is extremely thin throughout, and because of this the question may be raised as to whether *E. ellipsoidalis* Becker and Frye, 1929, is a valid species or only an abnormal form of E. *smithi.* The sporocysts are lemon-shaped, 14.4 to 16.2 by 7.2 to 7.5 microns in size. Each sporocyst incloses a moderately conspicuous residual body.

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[1] The numerator stands for length; the denominator for breadth.

EIMERIA BUKIDNONENSIS sp. nov. Plate 1, figs. 7, 8, and 9.

As mentioned above, this form was found together with *E. smithi* in the fæces of a bull from Bukidnon, Mindanao, that was shipped to Manila for slaughter. The oöcysts differ very markedly, both in appearance and in size, from those of the other coccidia harbored by cattle and other domesticated animals. The only species to which it bears some resemblance is *Eimeria ekdysios* Triffitt, 1928, described from a millipede. In view of this, the question may be asked as to whether or not this organism is a true parasite of cattle, for it is possible that the host animal might have ingested with its food a millipede or a related arthropod harboring the parasite. The possibility of a case of spurious parasitism seems remote, however, for the number of oöcysts found in the fæces is very large. This would hardly be the case had the animal in question ingested one or even several millipedes, considering that the contents of the digestive tract of a bull are very voluminous. Even if it should turn out that this coccidium is not a true parasite of cattle, it should still be considered a distinct species, for it differs from its closest relative, *E. ekdysios*, in its oöcysts being much larger and in lacking a definite residual body within the sporocysts.

The oöcysts are yellowish to darkish brown and are uniformly pyriform, the average shape index being 1.37. They measure 46.8 to 50.4 by 33.3 to 37.8 microns. The average size of twenty-five oöcysts is $\frac{48.6 \pm 0.156}{35.4 \pm 0.135}$ microns, with a standard deviation of $\frac{1.155 \pm 0.111}{1.002 \pm 0.095}$ and a coefficient of variation of $\frac{2.375 \pm 0.227}{2.825 \pm 0.269}$. The oöcyst wall shows radial striations and is about 2 microns thick except at the micropyle end where it is very thin. The micropyle is conspicuous, being about 4 microns wide. The sporocysts are lemon-shaped and 14.4 to 21.6 by 9.0 to 11.7 microns in size (average, 19.2 by 10.1 microns). The sporozoites are more or less roundish or reniform. A definite residual body has not been seen either inside the oöcyst or in the sporocysts.

COCCIDIUM FROM SHEEP AND GOATS

Two species of coccidia have been reported from sheep; namely, *Eimeria faurei* (Moussu and Marotel, 1901) and *E. intricata* Spiegl, 1925. The oöcysts of *E. faurei* are described as being ovoid and measuring 20 to 40 by 17 to 26 microns; those of *E. intricata* are larger, 42 to 60 by 31 to 44 microns, and possess rough surfaces. Both forms show micropyles that are closed by distinct caps.

From goats only one species, *E. arloingi* Marotel, 1905, has been reported. The oöcysts measure 21 to 33 by 16.5 to 22.5 microns. It is believed, however, especially by Nöller, Schürjohann, and Vorbrodt (1922), who carried out cross-infection experiments with sheep and goats, that this form is identical with *E. faurei* of sheep.

EIMERIA FAUREI (Moussu and Marotel, 1901). Plate 2, figs. 1 and 2.

This appears to be a common parasite of Philippine sheep and goats. The oöcysts seen in both types of hosts are so similar that there is no question as to their representing the same species. They are ovoid, with an average shape index of 1.37. In the fresh state they possess a delicate yellowish tinge. They measure 25.2 to 39.6 by 18.0 to 25.2 microns. The average size of twenty-five oöcysts from a goat is $\frac{29.0 \pm 0.498}{21.1 \pm 0.294}$ microns, with a standard deviation of $\frac{3.695 \pm 0.351}{2.178 \pm 0.208}$ and a coefficient of variation of $\frac{12.715 \pm 1.257}{10.322 \pm 1.010}$. The oöcyst wall is about 0.8 micron in thickness and is provided at the micropyle end with a sort of bulging cap. The sporocysts are lemon-shaped, measuring 10.8 to 14.4 by 7.2 to 9.0 microns. A residual body is inclosed within each sporocyst.

COCCIDIUM FROM SWINE

Two types of coccidia of the genus *Eimeria* have been recorded from swine. One type produces larger oöcysts, measuring 50.0 by 35.0 microns, while another type produces smaller oöcysts, 18.0 to 24.0 by 15.0 to 20.0 microns in size. Opinion is divided among different observers as to the significance of these two kinds of oöcysts. According to Douwes (1921) and others, it is possible that they represent distinct species. Nöller and Frenz (1922) and Biester and Murray (1929) believe, however, that they belong to a single species, because of the occurrence of oöcysts of intermediate size.

In the fæces of Philippine swine three types of oöcysts have been encountered, as follows: Larger ones, 23.0 to 30.0 by 18.0 to 23.0 microns; smaller ones, 18.0 to 19.0 by 14.0 to 16.0 microns; and intermediate forms, 19.8 to 23.4 by 19.0 to 21.0 microns, in size. With such figures as these and without any knowledge of the complete life cycles of the three types of oöcysts, it is really not permissible to separate them into distinct species. There are, however, other criteria by means of which they may be differentiated and these include, among others, the shape of the oöcysts, the thickness of the oöcyst wall, the shape and size of the sporocysts, the shape and size of the sporozoites, and the presence and structure of residual masses of protoplasm. These structures have been taken into consideration in the study of a large number of oöcysts; and it has been found that the three types, except for their differences in size, resemble one another most closely. They are, therefore, taken to represent only a single species; namely, *Eimeria debliecki* Douwes, 1921.

The importance of this parasite has been either minimized or entirely overlooked; but, according to Biester and Murray, it is often the cause of intestinal disturbances in swine in the United States. In Philippine pigs it is very common and it would seem advisable to keep it in mind in the diagnosis of obscure cases of diarrhœa that are often observed in native animals.

EIMERIA DEBLIECKI Douwes, 1921. Plate 2, figs. 3 and 4.

The oöcysts are either oval, ellipsoidal, or nearly spherical, with an average shape index of 1.35. They measure 17.1 to 32.4 by 12.6 to 23.4 microns. The average size of forty oöcysts is $\frac{25.5 \pm 0.497}{18.9 \pm 0.375}$ microns, with a standard deviation of $\frac{4.809 \pm 0.351}{3.513 \pm 0.265}$ and a coefficient of variation of $\frac{18.829 \pm 1.52}{18.528 \pm 1.48}$. The oöcyst wall is uniform in thickness, being 0.8 to 1.4 microns. The sporocysts are lemon-shaped and capped at their rostrated ends. They measure 9.0 to 18.0 by 5.4 to 7.2 microns. A roundish, coarsely granular residual body is present within each sporocyst.

COCCIDIA FROM DOGS AND CATS

According to Wenyon (1926), dogs and cats in various parts of the world are parasitized with at least five different kinds of coccidia; namely, *Isospora bigemina* (Stiles, 1891), *I. rivolta* (Grassi, 1879), *I. felis* Wenyon, 1923, *Eimeria canis* Wenyon, 1923, and *E. felina* Nieschultz, 1924. The status of the first three species has been questioned, although they are said to differ not only in size but also as regards their localization in the tissues of the intestinal villi of their hosts. Wenyon and Sheather

(1925) give the dimensions of their oöcysts and sporocysts as follows:

Species.	Oöcysts.	Sporocysts.
	Microns.	*Microns.*
Isospora felis	39.0–48.0 by 26.0–37.0	20.0–27.0 by 18.0–21.0
Isospora rivolta	20.0–24.0 by 15.0–20.0	12.0–15.0 by 9.0–10.0
Isospora bigemina (large)	18.0–20.0 by 14.0–16.0	18.5–15.5 by 9.0–10.0
Isospora bigemina (small)	10.0–16.0 by 7.5–10.0	7.5–10.0 by 5.0– 8.0

In Philippine dogs and cats, one kind of *Eimeria* and two types of *Isospora* oöcysts have been seen. There can be no doubt that the *Eimeria* oöcysts that were recovered from a dog are those of *E. canis* and the *Isospora* oöcysts from the pup that died of coccidiosis, those of *I. rivolta.* On the other hand, the *Isospora* oöcysts from the two cats already referred to are difficult to determine, since they are intermediate in size between *I. felis* and *I. rivolta* (see Table 1). The smaller ones may be those of *I. rivolta,* but those measuring 27.0 to 30.6 by 23.4 to 27.0 microns do not appear to be either *felis* or *rivolta.* They may possibly represent either a new species or a new variety of either *felis* or *rivolta.* Their identification must wait until their complete development has been worked out.

TABLE 1.—*Measurements in microns of Isospora oöcysts from two cats.*[a]

Length.	Breadth.							Total.
	19.8	21.6	22.5	23.4	25.2	26.1	27.0	
21.6	1							1
25.2		1	2	1				4
26.1		2						2
27.0		2		12				14
28.0			9	11	2			22
28.8				2	1			3
30.6						2	2	4
Total	1	5	11	26	8	2	2	50

[a] Range $\frac{21.6 - 30.6}{19.8 - 27.0}$ microns. Mean $\frac{27.5 \pm 0.139}{23.3 \pm 0.142}$ microns. Standard deviation $\frac{1.462 \pm 0.098}{1.491 \pm 0.100}$. Coefficient of variation $\frac{5.316 \pm 0.358}{6.399 \pm 0.431}$.

Haughwout (1918) has recorded the finding of coccidial oöcysts in a native cat under the name *Isospora bigemina.* That was at the time when the coccidia of dogs and cats were considered to represent only a single species. In view of their large size, 29.0 to 38.0 by 22.0 to 29.0 microns, it is possible that Haughwout was dealing with the oöcysts of *I. felis*.

EIMERIA CANIS Wenyon, 1923. Plate 2, figs. 7 and 8.

The oöcysts are oval, with an average shape index of 1.28. They measure 27.0 to 34.2 by 21.6 to 25.2 microns. The average size of twenty-five oöcysts is $\frac{30.4 \pm 0.282}{23.7 \pm 0.161}$ microns, with a standard deviation of $\frac{2.091 \pm 0.200}{1.196 \pm 0.114}$ and a coefficient of variation of $\frac{6.878 \pm 0.660}{5.046 \pm 0.484}$. The oöcyst wall is uniform in thickness and is usually provided with a visible micropyle. It incloses a large globular body which may be of the nature of a residual body. The sporocysts are lemon-shaped, 14.4 to 16.2 by 7.2 to 9.0 microns in size, and each incloses a roundish and coarsely granular residual body.

ISOSPORA RIVOLTA (Grassi, 1879). Plate 2, figs. 9 and 10.

The oöcysts are usually ovoidal, but sometimes they are ellipsoidal or nearly spherical; the average shape index is 1.23. They measure 18.0 to 23.4 by 15.7 to 19.8 microns. The average size of twenty-five oöcysts is $\frac{20.8 \pm 0.250}{16.7 \pm 0.169}$ microns, with a standard deviation of $\frac{1.852 \pm 0.176}{1.253 \pm 0.119}$ and a coefficient of variation of $\frac{8.869 \pm 0.846}{7.476 \pm 0.713}$. The oöcyst wall is uniformly thin and delicate and is easily distorted. The sporocysts, of which there are four, instead of two as in the members of the genus *Eimeria*, are roundish or ellipsoidal and measure 12.6 to 14.4 by 9.0 to 10.8 microns. Each sporocyst contains four club-shaped sporozoites and a large, rounded, granular, residual body.

COCCIDIUM FROM CHICKENS

Chickens have usually been thought to harbor only one species of coccidium to which the name *Eimeria avium* has been given. According to Tyzzer (1929), this name has been erroneously credited to Rivolta and Silvestrini (1873) and it was Rivolta (1878) alone who referred to this pathogenic coccidium of fowls under the designation *Gregarina avium intestinalis*. The latter name, being a trinomial, is however, not valid in a nomenclatural sense; hence, Tyzzer has proposed to adopt in its place the name *Eimeria tenella* (Railliet and Lucet, 1891). It also transpires from Tyzzer's investigations that instead of a single species there are four species of chicken coccidia; namely, *E. tenella* (Railliet and Lucet, 1891), *E. mitis* Tyzzer, 1929, *E. acervulina* Tyzzer, 1929, and *E. maxima* Tyzzer, 1929. These are said to differ among themselves not only in size but also in the character and location of the gross lesions that they produce in the intestines of their hosts, as shown in Table 2.

TABLE 2.—*Coccidia of chickens and their differentiation (after Tyzzer).*

Coccidium.	Size of oöcyst.	Character and location of gross lesions.
	Microns.	
Eimeria tenella	$\frac{19.6\text{–}26.1}{16.3\text{–}22.8}$	At onset, hæmorrhage into cæca and lower intestine. Later thickening, milky whitening of cæcal mucosa, and cores of clotted blood.
Eimeria mitis	$\frac{14.3\text{–}19.6}{13.0\text{–}17.0}$	None.
Eimeria acervulina	$\frac{17.7\text{–}20.2}{13.7\text{–}16.3}$	Opaque grayish spots in upper intestine representing collections of oöcysts. When more extensive, the infected areas coalesce so that mucosa appears mottled.
Eimeria maxima	$\frac{21.4\text{–}42.5}{16.5\text{–}29.8}$	Thickening of small intestine most pronounced in middle portion. Blood-tinged exudate or small hæmorrhages.

So far oöcysts of only one type have been encountered in the fæces of native fowls. In view of their size, they have been identified as *E. tenella.*

EIMERIA TENELLA (Railliet and Lucet, 1891). Plate 2, figs. 5 and 6.

According to Tyzzer, this is the most pathogenic species of chicken coccidium. The oöcysts are generally ovoid or subspherical, with an average shape index of 1.11. In the fresh state they are either colorless or possess a greenish or yellowish tinge, probably depending upon the color of the intestinal contents. They measure 18.0 to 21.6 by 14.4 to 21.6 microns. The average size of twenty-five oöcysts is $\frac{19.8 \pm 0.202}{17.7 \pm 0.274}$ microns, with a standard deviation of $\frac{1.673 \pm 0.143}{2.263 \pm 0.194}$ and a coefficient of variation of $\frac{8.449 \pm 0.725}{12.785 \pm 1.128}$. The oöcyst wall is uniform in thickness and contains at its micropyle end a sort of plug that is quite conspicuous in some oöcysts. Within the oöcyst there is generally found a small globule, the nature of which is not known. The sporocysts are oval, rostrated at one end, and measure 9.0 to 11.0 by 5.5 to 7.0 microns. The sporozoites are reniform. A definite residual body has not been seen within the sporocysts.

DIFFERENTIATION OF COCCIDIAL OÖCYSTS

One other object of the survey, besides the enumeration of the coccidial parasites of Philippine domesticated animals, was to ascertain if the oöcysts of the various species of these organisms could be so differentiated from one another morphologically as to enable one to recognize them individually if they were mixed together or if encountered in animals other than their respective proper hosts; that is, the ones to which they are respectively best adapted. The reason for this is the uncertainty that prevails regarding the identity of some of the coccidia of domesticated animals and of man. Some parasitologists, who consider these parasites as nonspecific in the sense that they are interchangeable among a variety of different hosts, believe, for example, that the coccidia of man and of cats, dogs, and rabbits are identical. The same view is held with respect to those of cattle and swine. Other parasitologists, on the other hand, who maintain that these organisms are specific in the sense that a certain species of coccidium can infect only a single kind of host, or, at most, only a group of very closely related host species, believe that the coccidia of the different animals, including those of man, represent separate species.

It is of the utmost importance, as pointed out by Andrews (1927), to be informed definitely if these organisms are specifically related or not to their respective proper hosts, for we should know whether or not man is endangered by the coccidial parasites infecting his livestock and household pets, and also whether or not the latter are susceptible to the coccidioses that are common among many wild animals. Many attempts have been made to solve this problem by means of cross-infection experiments, the most recent being those of Uhlhorn (1926), Andrews (1927), and Corcuff (1928). Although many of these experiments (Andrews; Corcuff) have yielded results in favor of the specificity hypothesis, a few of them (Uhlhorn) have yielded just the opposite results, so that the question cannot be considered as definitely settled. It may be said in connection with cross-infection experiments that the results may be misleading unless, as also pointed out by Andrews, the following factors are taken into consideration: (*a*) The natural coccidial parasites of, (*b*) the interference of an acquired immunity by, and (*c*) the mechanical carriage of coccidial oöcysts by, the animals used in the experiments. The confusion that may result from these factors might be guarded against, however, if, in addition to employing only young and apparently noninfected animals in the experiments, one could distinguish morphologically the different kinds of coccidial oöcysts.

The morphological differentiation of coccidial oöcysts is an easy matter in some cases, but in others it may be difficult or even impossible without a full knowledge of their life histories. It is, of course, easy to distinguish the fully developed oöcysts of the members of the genus *Isospora* from those of the genus *Eimeria* by the number of sporocysts within each oöcyst and the number of sporozoites within each sporocyst, the isosporæ being disporocystid and tetrazoic and the eimeriæ being tetrasporocystid and dizoic. Among members of the same genus also differentiation may not be difficult, due to the possession by some species of characteristic features that readily mark them off from other species. For example, the caplike structure at the micropyle end of the oöcyst of *Eimeria faurei* and the very conspicuous micropyle of the deeply stained oöcyst of *E. bukidnonensis* at once separate these parasites of goats and sheep and of cattle, respectively, from those of the other domesticated animals. In other instances, however, as in the case of the coccidia of poultry and of cats and dogs, identification may be very difficult due to apparent morphological resemblances. In these cases a determination based on shape and size, as had often been done formerly, cannot be relied upon exclusively; otherwise, if an overlapping of sizes should occur, one would be led to consider as identical organisms that in reality represent separate species. The converse of this may also happen; that is, the oöcysts of a single species may be taken to represent two or more species, as in the case of *Eimeria debliecki* of the pig and of *E. lacazie* of the English sparrow, in which, as the recent observations of Boughton (1930) seem to indicate, there is a wide variation in the size of the oöcysts. In addition to (*a*) shape and (*b*) size, it is, therefore, necessary to note other factors, such as the following: (*c*) The thickness and appearance of the oöcyst wall; (*d*) the number, appearance, and size of the sporocysts; (*e*) the number and structure of the sporozoites; (*f*) the presence or absence and the structure, if present, of any residual protoplasm within the oöcyst after the formation of the sporoblasts (oöcystic residual body); and (*g*) the presence or absence and the structure, if present, of any residual protoplasm after the development of the sporozoites (sporocystic residual body).

These structures have been taken into consideration in the determination of the coccidial oöcysts dealt with in this paper. In Table 3 are presented the distinguishing features of the various species. By reference to the table it will be seen that, with the exception of the isosporan oöcysts of the dog and cat and of *Eimeria smithi* and *E. debliecki*, their differentiation is comparatively easy. As already stated, *E. faurei* of sheep and goats is readily distinguished by the possession of a caplike protuberance at the micropyle end of the oöcyst wall and *E. bukidnonensis* by the shape and size of the oöcysts and the presence of a very conspicuous micropyle. *Eimeria canis* of the dog bears a slight resemblance to *E. debliecki* of the pig, especially in the great range of its size, as reported by Wenyon (1923), but it may be distinguished from the latter by the presence of a more or less distinct micropyle and of a large globular body, which may be of the nature of an oöcystic residual body. *Eimeria zürnii* of cattle and *E. tenella* of chickens may also be mistaken for one another by their shape and size and by the absence in both of definite sporocystic residual bodies, but they may be differentiated by the presence in *E. tenella* of a plugged micropyle and of a small globular body inside the oöcyst. In the case of *E. smithi* of cattle and carabaos and *E. debliecki* of swine the similarity is greater. A very close examination of their sporocysts, however, will disclose the fact that those of *E. debliecki* are capped at their rostrated ends, while those of *E. smithi* are not (Plate 1, fig. 7; Plate 2, fig. 5). In this feature alone and in the fact that the oöcyst wall of *E. smithi* is very thin at the micropyle end while that of *E. debliecki* is more or less uniform in thickness throughout, do the two forms constantly differ. In other respects they resemble each other so closely that it is no wonder that several observers have considered them as belonging to a single species. With reference to the isosporan oöcysts, it is possible, as stated above, that they represent two separate species, but only with a full knowledge of their complete life histories could one definitely determine whether they are distinct or not.

TABLE 3.—*Comparison of coccidial oöcysts from different kinds of domesticated animals.*

Species.	Host.	Oöcyst.				Sporocyst.		
		Thickness of oöcyst wall.	Shape and shape index.	Size.	Residual body.	Shape.	Size.	Residual body.
		Microns.		*Microns.*			*Microns.*	
Eimeria zürnii	Cattle; carabao	1.1; uniform	Spherical to ovoidal; 1.10.	$\frac{13.5\text{–}20.2}{12.3\text{–}18.0}$	None	Lemon-shaped	$\frac{7.8\text{–}11.2}{5.1\text{–}\ 5.8}$	None.
Eimeria bukidnonensis	Cattle	2; much thinner at micropyle end; micropyle 4 μ across.	Pyriform; 1.37	$\frac{46.8\text{–}50.4}{33.3\text{–}37.8}$	do	do	$\frac{14.4\text{–}21.6}{9.0\text{–}11.7}$	Do.
Eimeria smithi	Cattle; carabao	1.3; thinner at micropyle end.	Oval; 1.51	$\frac{28.8\text{–}39.6}{19.8\text{–}27.0}$	do	do	$\frac{14.4\text{–}16.2}{7.2\text{–}\ 7.5}$	Present.
Eimeria debliecki	Pig	0.8–1.5; nearly uniform.	Oval; 1.35	$\frac{17.1\text{–}32.4}{12.6\text{–}23.4}$	do	do	$\frac{9.0\text{–}18.0}{5.4\text{–}\ 7.2}$	Do.
Eimeria faurei	Sheep; goat	0.8; micropyle covered with a bulging cap.	Oval; 1.37	$\frac{25.2\text{–}39.6}{18.0\text{–}25.2}$	do	do	$\frac{10.8\text{–}14.4}{7.2\text{–}\ 9.0}$	Do.
Eimeria canis	Dog	1.2; uniform, with distinct micropyle.	Oval; 1.28	$\frac{27.0\text{–}34.2}{21.6\text{–}25.2}$	Globular body present.	do	$\frac{14.4\text{–}16.2}{7.2\text{–}\ 9.0}$	Do.
Eimeria tenella	Chicken	1; uniform; plugged micropyle.	Spherical to oval; 1.11.	$\frac{18.0\text{–}21.6}{14.4\text{–}21.6}$	Small globular body often present.	do	$\frac{9.0\text{–}11.0}{5.5\text{–}\ 7.0}$	None.
Isospora rivolta	Dog	0.7; uniform	Oval; 1.23	$\frac{18.0\text{–}23.4}{15.7\text{–}19.8}$	None	Roundish ellipsoidal.	$\frac{12.6\text{–}14.4}{9.0\text{–}10.8}$	Present.
Isospora sp	Cat	0.9; uniform	Oval; 1.17	$\frac{21.6\text{–}30.6}{19.8\text{–}27.0}$	do	do	$\frac{14.4\text{–}18.0}{10.8\text{–}13.5}$	Do.

SUMMARY

1. Cases of coccidiosis in cow and carabao calves and in pups are reported; also in two adult cats which were apparently only carriers of coccidial parasites, but which came down with acute symptoms of coccidiosis when infected experimentally with surra trypanosomes (*Trypanosoma evansi*).

2. Presuming that young animals derive their infections from their infected mothers and other adult animals that play the rôle of carriers, a survey was made among the different domesticated animals and the oöcysts of the following coccidial parasites were encountered: *Eimeria zürnii, E. smithi,* and a new species, *E. bukidnonensis,* in cattle; *E. zürnii* and *E. smithi* in carabaos; *E. faurei* in sheep and goats; *E. debliecki* in swine; *E. canis* and *Isospora rivolta* in dogs; *Isospora* sp. in cats; *E. tenella* in chickens.

3. These various coccidia appear to be morphologically distinct and the differentiation of their oöcysts is facilitated by means of a table.

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ILLUSTRATIONS

[Unless stated otherwise, the magnification of the figures is × 1120.]

Plate 1

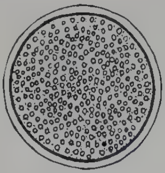
FIG. 1. *Eimeria zürnii,* young oöcyst; zygote fills entire space within oöcyst wall.

2. *Eimeria zürnii,* oöcyst in which the zygote has contracted preparatory to division.
3. *Eimeria zürnii,* oöcyst with four sporoblasts.
4. *Eimeria zürnii,* mature oöcyst with four sporocysts each of which incloses two sporozoites.
5. *Eimeria smithi,* oöcyst in which the zygote has contracted preparatory to division.
6. *Eimeria smithi,* mature oöcyst with four sporocysts each inclosing two sporozoites.
7. *Eimeria smithi,* enlarged drawing of sporocyst showing details, × 1840.
8. *Eimeria bukidnonensis,* oöcyst in which the zygote has contracted preparatory to division.
9. *Eimeria bukidnonensis,* oöcyst with four sporoblasts.
10. *Eimeria bukidnonensis,* mature oöcyst with four sporocysts each inclosing two sporozoites.

Plate 2

FIG. 1. *Eimeria faurei,* oöcyst in which the zygote has contracted preparatory to division.

2. *Eimeria faurei,* mature oöcyst with four sporocysts each inclosing two sporozoites.
3. *Eimeria debliecki,* oöcyst in which the zygote has contracted preparatory to division.
4. *Eimeria debliecki,* mature oöcyst with four sporocysts each inclosing two sporozoites.
5. *Eimeria debliecki,* enlarged drawing of sporocyst showing details, × 1840.
6. *Eimeria tenella,* oöcyst in which the zygote has contracted preparatory to division.
7. *Eimeria tenella,* mature oöcyst with four sporocysts each inclosing two sporozoites.
8. *Eimeria canis,* oöcyst in which the zygote has contracted preparatory to division.
9. *Eimeria canis,* mature oöcyst with four sporocysts each inclosing two sporozoites.
10. *Isospora rivolta,* oöcyst in which the zygote has contracted preparatory to division.
11. *Isospora rivolta,* mature oöcyst with two sporocysts each inclosing four sporozoites.



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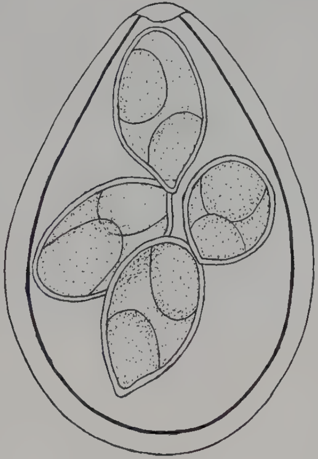

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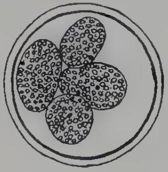

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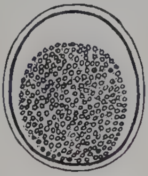

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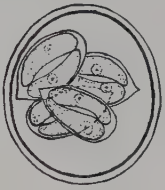

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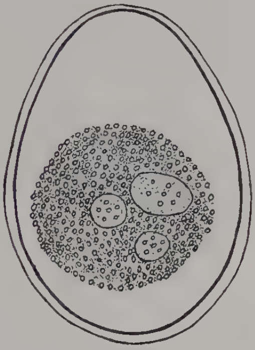

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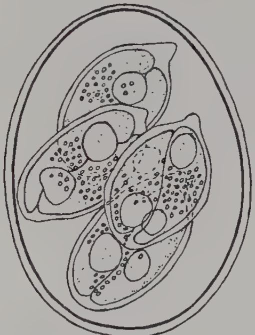

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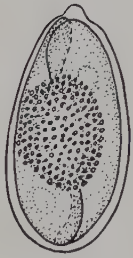

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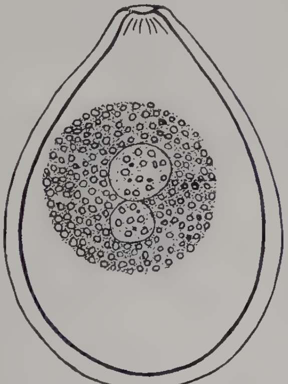

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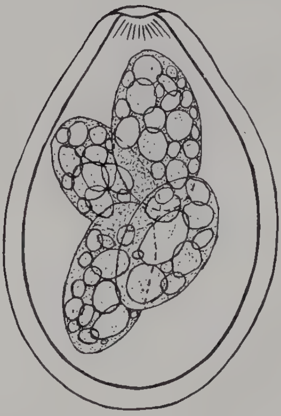

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PLATE 1.

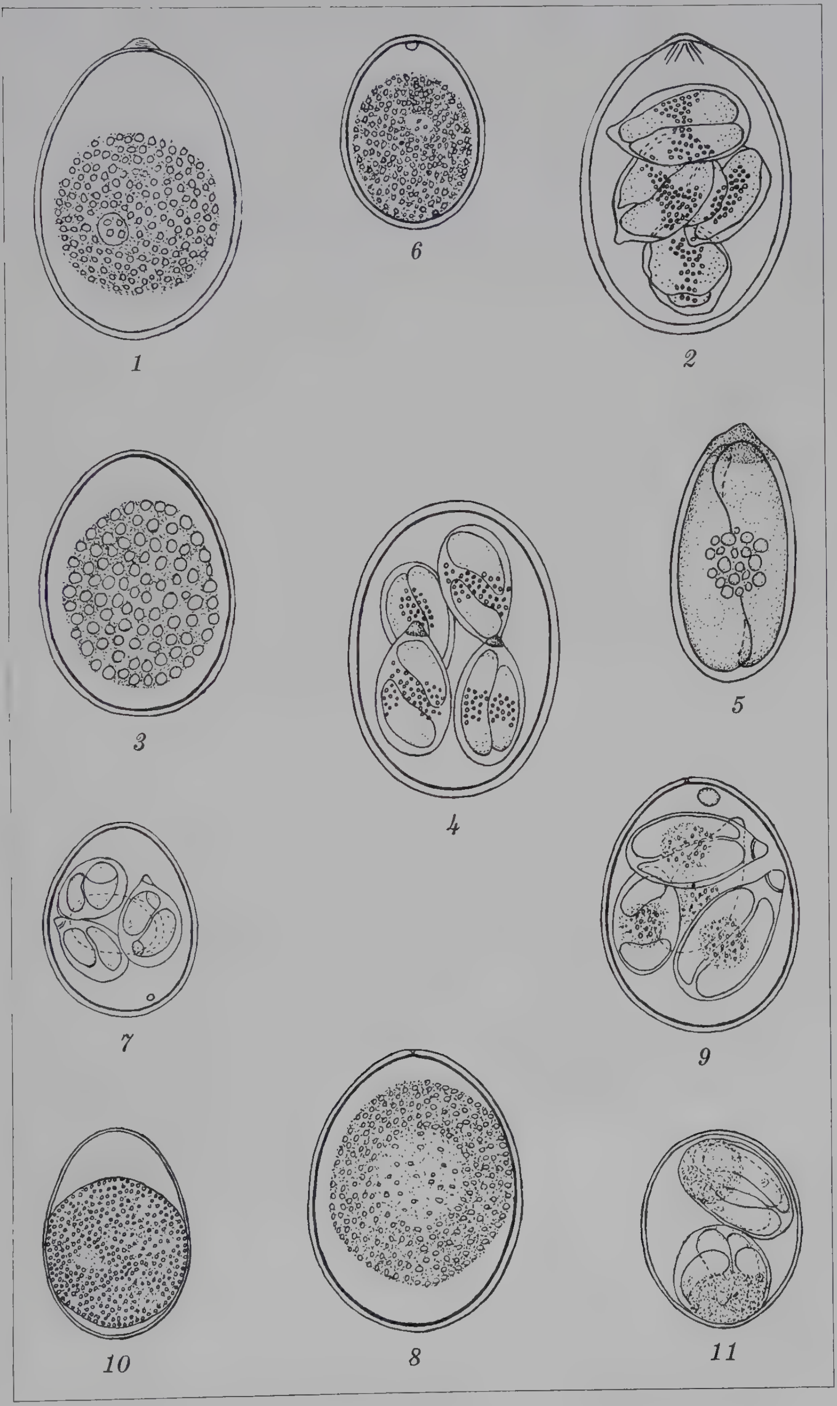
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PLATE 2.

TREMATODE PARASITES OF PHILIPPINE VERTEBRATES, II: TWO ECHINOSTOME FLUKES FROM RATS

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TWO PLATES

In 1928, the writer published a descriptive list of trematodes parasitic in Philippine vertebrates. It is the intention to amplify the list from time to time as other related parasites are encountered and identified. The practice adopted of recording the individual worms under their respective hosts will be adhered to. The present paper has to do with two echinostomes found in the small intestine of wild rats, *Mus norvegicus* Erxleben, 1777 (=*M. decumanus* Pallas, 1778). Later, when the number recorded has reached a certain limit, the systematic arrangement of the different forms may be attempted.

The rats from which the parasites were recovered were among those trapped in the City of Manila by representatives of the Philippine Health Service and brought to the Bureau of Science for routine bubonic-plague inspection. Usually many of these rodents are brought in every day, of which three or four are opened for the detection of intestinal and other parasites. Of one hundred nine rats thus far examined, one harbored twenty-one specimens of an echinostome that is believed to be identical with the human intestinal fluke, *Euparyphium ilocanum*=*Echinostoma ilocanum* (Garrison, 1908); another rat yielded over one hundred specimens of an apparently new species of the genus *Euparyphium.*

This report brings the number of recorded species of flukes infesting the brown rat to a total of fourteen. Dollfus (1925), who has reviewed the literature dealing with the parasitic distomes of rats in general, assigns the following to *Mus norvegicus: Echinostoma cinetorchis* Ando and Ozaki, 1923; *E. gotoi* Ando and Ozaki, 1923; *E. macrorchis* Ando and Ozaki, 1923;

E. spiculator (Dujardin, 1843); *Echinoparyphirum japonicum* Ando and Ozaki, 1923; *Heterechinostoma magnovatum* Stunkard and Haviland, 1924; *Clonorchis sinensis* (Cobbold, 1875); *Lepoderma muris* Tanabe, 1922; *Ascocotyle* (*Parascocotyle*) *diminuta* Stunkard and Haviland, 1924; and *Distoma* sp. Podiapolsky, 1924. To these should be added *Ascocotyle arnaldoi* Travassos, 1928, and *Pygidiopsis macrostomum* Travassos, 1928. Among these *Clonorchis sinensis* is a common parasite of human beings in certain regions of China and Japan, while *Echinostoma macrorchis* has been reported once in man by Majima (1927).

EUPARYPHIUM ILOCANUM (Garrison, 1908) Tubangui, 1931. Plate 1, figs. 1, 2, 3, 4.

The adult form of this parasite was first recovered by Garrison (1908) from the stools of Filipino (Ilocano) prisoners in Bilibid Prison, Manila, after the administration of male fern. Garrison believed it represented a new genus and a new species of fluke and called it *Fascioletta ilocana.* Odhner (1911), however, who received four specimens from Garrison, discovered the presence of a spined cephalic collar on one of the specimens and thereby established its position in the old genus *Echinostoma* Rudolphi, 1809. The latest available morphological account of the worm is that by Hilario and Wharton (1917), who not only confirmed Odhner's observation on the presence of a cephalic collar but also contributed other details to its description.

Compared with the specimens of human origin, as described by these various authors, those obtained from the rat appear to differ in the size of the acetabulum, the average length of the œsophagus, the diameter of the cephalic collar, and in other minor features that are liable to occur among members of one species (see Table 1). In their more important characters, they tally with the description of *Echinostoma ilocanum,* except in the nature and extent of the armature of the cuticle and in the number and size of the collar spines. With regard to these structures, however, it should be remembered that the previous descriptions of the parasite were based on specimens obtained after anthelmintic medication. Specimens so obtained must have been in a macerated or partly macerated condition and, very likely, must have lost part or all of the spines on the head collar and on the rest of the body surface. Hilario and Wharton pointed out that these structures are very unstable and easily lost. An examination of the material at hand corroborates this statement, for, of the twenty-one specimens from the rat, three appear to have undergone a certain degree of maceration and are without any scales or spines and the cephalic collar is not evident. These three specimens resemble very closely those that the writer had occasion to collect from the stools of students after the administration of carbon tetrachloride (Tubangui and Francisco, 1925).

This parasite has previously been reported only in natives from Zambales and Ilocos Sur Provinces, Luzon. Its presence in Manila rats is interesting from the standpoint of geographical distribution, for it sustains the view of Hilario and Wharton that the fluke may prove to be found in other places in the Philippine Islands. Whether originally it has had a wider field of distribution or whether it has only been introduced into Manila and other places, is not known. It is certain, however, that the molluscan intermediate host occurs, at least, in Manila, otherwise the parasite could not have found its way into the body of a rat (that is, unless the rat in question was brought to Manila on an interisland steamer from either Zambales or Ilocos Sur). If this be the case, the apparent absence of the worm in other Filipinos may be explained by differences of habit, particularly in the selection and preparation of foods.

In the following description it will be observed that the morphology of the trematode is such that it may be assigned either to the genus *Echinostoma* or to the genus *Eupharyphium*, depending upon what should be considered as the most important distinguishing character of each genus. As defined by Stiles and Hassall (1926), these two genera possess many features in common, differing only in the following respects: In *Echinostoma* the spines of the skin are confined to the ventral surface of the forebody and the uterus is long, with many loops and eggs; in *Eupharyphium* the spines of the skin are found on nearly the entire ventral surface of the body and on the extreme anterior portion of the dorsal surface, and the uterus is short and contains only a few eggs. The fluke in question conforms with the definition of *Echinostoma* in the length of its uterus and in the number of eggs present and with that of *Eupharyphium* in the distribution of its cuticular spines. Conversely, it disagrees with the former genus in the extent of its cuticular spines and with the latter in the length of its uterus.

The length of the uterus, however, does not appear to be a very constant character, at least, in the genus *Echinostoma*, for there are forms like *E. lobulatum* Odhner, 1910 and *E. boleocephalum* (von Linstow, 1873), the uteri of which are short and contain only a few eggs. On the other hand, there are no

TABLE 1.—*Morphology of Euparyphium ilocanum according to different authors.*

Author.	Size.	Cuticle.	Size of oral sucker.	Position of acetabulum.
	mm.		*mm.*	
Garrison, 1908	4.0–6.0 by 0.75–1.35	Smooth	0.13–0.20 by 0.075–0.130	Between first and second fifths of body length.
Odhner, 1911	4.0–5.0 by 1.00–1.35	Covered with plaquelike scales as far caudad as posterior testis.	0.18 (diameter)	At about 0.7 mm from anterior end.
Hilario and Wharton, 1917	4.03–7.82 by 0.98–1.60	In some specimens anterior part of body covered with very small spines; spines seem very unstable and easily lost.	0.120–0.200 (diameter)	Middle of anterior third of body length.
Tubangui, the present paper	5.57–8.02 by 1.33–1.58	Covered, ventrally up to second testis or beyond and dorsally up to acetabulum, with flat scales.	0.19–0.24 (diameter)	Do.

Author.	Size of acetabulum.	Diameter of head collar.	Number of collar spines.	Size of pharynx.	Length of œsophagus.
	mm.	*mm.*		*mm.*	*mm.*
Garrison, 1908	0.48–0.52 (diameter)			0.15–0.19 (diameter)	0.05–0.10.
Odhner, 1911	0.40–0.46 (diameter)	0.30–0.33	49	0.17 by 0.11	Short (0.16 mm, according to Odhner's illustration).
Hilario and Wharton, 1917	0.53 (average diameter)		39	0.160 (average diameter)	0.05–0.12.
Tubangui, the present paper	0.60–0.69 by 0.64–0.74	0.34–0.46	51	0.19–0.20 by 0.15–0.17	0.10–0.20.

TABLE 1.—*Morphology of Euparyphium ilocanum according to different authors*—Continued.

Author.	Shape and position of ovary.	Shape of testes.	Anterior extent of vitellaria.	Size of cirrus sac.	Size of eggs.
				mm.	*Microns.*
Garrison, 1908	Globular; at center of body length, slightly to right of median line.	More or less distinctly divided by transverse circular constriction into anterior and posterior lobes or 4- to 5-lobed.	Slightly behind junction of anterior and middle thirds of body length.	0.560–0.608 by 0.240–0.280	88.8–114.7 by 53.5–81.9.
Odhner, 1911	Transversely compressed, median, in front of testis.	Deeply lobed	Short distance behind acetabulum.		92–114 by 58–82.
Hilario and Wharton 1917.	Globular; a little to right of median line, at about middle of body length.	Oval, but generally elongated and distinctly divided into anterior and posterior lobes by transverse constriction.	Midway between posterior border of acetabulum and anterior border of ovary.	Twice as long as wide	88.8–111.0 by 53.6–74.4
Tubangui, the present paper.	Globular, or slightly compressed transversely, median, preëquatorial, at about middle of second fourth of body length.	Elongate and divided into anterior and posterior lobes by transverse constriction or shorter and deeply indented into 3 or 4 lobes.	do	0.51–0.65 by 0.26–0.34	85.5–101.5 by 54.0–64.2

available indications as to the variability of the armature of the body surface among members of the two genera. It would appear, therefore, that the distribution of the cuticular spines constitutes a better criterion in differentiating between these two groups of echinostomes. For this reason the parasite from the rat has been assigned to the genus *Eupharyphium*.

DESCRIPTION

Body moderately large, elongate, grayish and opaque in appearance in the preserved condition, except at anterior end where it is whitish and more transparent; measures from 5.57 to 8.02 by 1.33 to 1.58 millimeters, maximum breadth at or near equator of body; anterior extremity attenuate, posterior end rounded. Lateral sides of body from anterior end to acetabulum rolled ventrally. Cuticle armed with flat scalelike structures (Plate 1, fig. 3), which are distributed ventrally from anterior end to second testis or slightly beyond that level, and dorsally from anterior end to level of anterior border of acetabulum; scales 13.5 to 24.7 by 13.5 to 18.0 microns in size, those at anterior end being smaller. Suckers close together; oral sucker small, subterminal, 0.19 to 0.24 millimeter in transverse diameter; acetabulum large, cup-shaped, at middle of anterior third of body length, 0.60 to 0.69 by 0.64 to 0.74 millimeter in size. Oral sucker surrounded dorsally and laterally by a collar (Plate 1, fig. 2) bearing fifty-one spines arranged in two alternating rows; collar 0.38 to 0.46 millimeter in diameter, reniform, its two rounded ventral angles united ventrally by a narrow ridge. Collar spines may be grouped as follows: Six ventral corner spines on each side of collar, the smallest of which measures 36.0 by 11.2 microns, the widest 42.7 by 15.7 microns, and the longest 45.0 by 11.2 microns; fourteen lateral spines on each side, arranged in pairs and eleven dorsal spines; lateral and dorsal spines 31.5 to 45.0 by 11.2 to 13.5 microns in size.[1]

Mouth terminal to subterminal, followed in some specimens by a very short prepharynx, 0.03 to 0.05 millimeter in length; pharynx 0.19 to 0.20 by 0.15 to 0.17 millimeter in size; œsophagus 0.10 to 0.20 millimeter long, bifurcating immediately in front of level of genital pore, midway between pharynx and acetabulum or slightly anterior of that level; intestinal cæca reach posteriorly to from 0.24 to 0.43 millimeter from posterior end of body.

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[1] Observations on arrangement and measurement of body scales and collar spines in this and the next species were made not on stained and dehydrated material but on compressed alcoholic specimens cleared in glycerin. It is rather difficult to measure and to count accurately the collar spines on stained specimens mounted in balsam.

Testes tandem, postequatorial, at third fourth of body length, either elongate and each divided into anterior and posterior lobes by transverse constriction or shorter and distinctly 3- or 4-lobed (Plate 1, fig. 4). Cirrus sac large, 0.51 to 0.65 by 0.26 to 0.34 millimeter in size, reaching to but not extending posteriorly beyond equator of acetabulum; incloses prominent seminal vesicle, well-developed pars prostatica, and protrusible cirrus. Common genital opening preacetabular, behind œsophageal bifurcation, to one side of median line.

Ovary globular or slightly compressed transversely, median, preëquatorial, usually behind middle of second fourth of body length, 0.31 to 0.43 by 0.34 to 0.48 millimeter in size; shell gland large, between ovary and anterior testis; receptaculum seminis absent, Laurer's canal present; uterus well-developed, occupying space bounded by ovary, acetabulum, and intestinal cæca. Vitellaria in moderately large follicles, commencing anteriorly on both sides at level about midway between posterior border of acetabulum and anterior border of ovary; anteriorly they are extracæcal, but behind second testis the follicles from the two sides unite and occupy most of posterior body region; transverse vitelline ducts and vitelline reservoir dorsal of shell gland, directly in front of anterior testis. Eggs numerous, operculated, light brown or yellowish, 85.5 to 101.5 by 54.0 to 65.2 microns in size.

Excretory system typical of echinostomes in general; excretory bladder long, with several small side branches, dividing into two principal branches behind second testis; excretory pore at extreme posterior end of body.

In view of the above anatomical findings, *Euparyphium ilocanum* should have the following—

Specific diagnosis.—Euparyphium: Body moderately large, elongate, 4.00 to 8.02 by 0.75 to 1.60 millimeters in size. Cuticle armed with flat scales, ventrally from anterior end to level of second testis or slightly beyond and dorsally from anterior end to level of acetabulum. Spines on cephalic collar fifty-one, grouped into six ventral corner and fourteen lateral spines on each side and eleven dorsal spines. Prepharynx very short;

œsophagus short to moderately long. Testes either elongate and divided by transverse constriction into anterior and posterior lobes or shorter and distinctly 3- to 4-lobed; cirrus sac large, reaching to but not beyond equator of acetabulum. Ovary globular or transversely oval; uterus well developed; eggs 85.5 to 114.7 by 53.5 to 82.0 microns in size.

Hosts.—Man, rat (*Mus norvegicus*).

Location.—Small intestine.

Localities.—Zambales, Ilocos Sur, Manila (? Ilocos Norte), Luzon.

EUPARYPHIUM GUERREROI sp. nov. Plate 2, figs. 1, 2, 3, 4.

This fluke differs from the echinostomes that have so far been reported from rats by the number of its collar spines, of which there are fifty-five. I take the pleasure of naming it in honor of Dr. Luis Guerrero, who, some years ago during a conversation, mentioned having encountered an echinostome in the intestine of a rat and who, therefore, was partly responsible in directing my attention to a study of the helminths infesting rats.

DESCRIPTION

Body slender, elongate, measuring 2.92 to 4.03 by 0.37 to 0.50 millimeters; maximum breadth across acetabulum or anywhere between that organ and anterior testis. Cuticle armed with flat scales, dorsally from anterior end to level of acetabulum and ventrally from anterior end to posterior testis or slightly beyond that level; scales (Plate 2, fig. 3) 6.0 to 15.0 by 5.5 to 9.4 microns, anterior ones being smaller. Oral sucker weak, subterminal, 0.10 to 0.12 millimeter in transverse diameter; acetabulum more powerful, at middle of anterior third of body length, 0.27 to 0.36 by 0.31 to 0.34 millimeter in size. Oral sucker surrounded dorsally and laterally by a collar bearing fifty-five spines arranged in two alternating rows; collar (Plate 2, fig. 2) 0.22 to 0.26 millimeter in diameter, reniform, its two ventral angles united by a narrow ridge. Collar spines may be grouped as follows: Five ventral corner spines on each side of cephalic collar, 24.7 to 31.5 by 9.0 to 11.9 microns; fifteen lateral spines on each side, 27.0 to 29.2 by 9.0 microns; and fifteen dorsal spines, 11.2 to 13.5 by 6.7 to 9.0 microns in size.

Mouth subterminal to terminal, succeeded by prepharynx 0.03 to 0.07 millimeter long; pharynx 0.10 to 0.11 by 0.07 to 0.08 millimeter in size; œsophagus 0.08 to 0.15 millimeter long, bifurcating in front of level of genital pore; intestinal cæca long, narrow in diameter, reaching to from 0.21 to 0.24 millimeter from posterior end of body.

Testes (Plate 2, fig. 4) tandem, postequatorial, at third fourth of body length, oval or sausage-shaped, often transversely constricted into anterior and posterior lobes; anterior testis usually smaller, at least shorter, 0.19 to 0.36 by 0.15 to 0.22 millimeter; posterior testis 0.27 to 0.39 by 0.12 to 0.20 millimeter in size. Cirrus pouch oval, 0.17 to 0.27 by 0.10 to 0.13 millimeter in size, immediately behind œsophageal bifurcation, not reaching posteriorly beyond equator of acetabulum; incloses large seminal vesicle, moderately developed pars prostatica, and protrusible cirrus; cirrus aspinose. Common genital opening preacetabular, behind œsophageal bifurcation, to one side of median line.

Ovary globular or slightly compressed; 0.10 to 0.15 by 0.07 to 0.13 millimeter in size, directly preëquatorial, pretesticular; shell gland prominent, filling most of the space between ovary and anterior testis; receptaculum seminis absent, Laurer's canal present; uterus short, with few coils, between ovary and acetabulum. Vitellaria in small or moderately large follicles, commencing anteriorly at middle of second fourth of body length, those on left side usually commencing at a more posterior level; behind second testis follicles from two sides unite and extend to posterior end of body; transverse vitelline ducts and vitelline reservoir dorsal of shell gland and immediately in front of second testis. Eggs few, operculated, thin shelled, light brown or yellowish, 78.7 to 85.5 by 54.0 to 60.7 microns in size.

Excretory system of usual echinostome type; excretory bladder long, tubular, dividing into two branches behind second testis; excretory pore at extreme posterior end of body.

Specific diagnosis.—Euparyphium: Body small, slender, 2.92 to 4.03 by 0.37 to 0.50 millimeters in size. Cuticle armed with flat scales, 6.0 to 15.0 by 5.5 to 9.4 microns in size. Head collar 0.22 to 0.26 millimeter in diameter, with fifty-five spines arranged in two alternating rows and grouped as follows: Five ventral corner and fifteen lateral on each side of collar and fifteen dorsal spines. Prepharynx and œsophagus 0.03 to 0.07 and 0.08 to 0.15 millimeter long, respectively. Testes postequatorial, unequal, oval or sausage-shaped, often constricted in middle into anterior and posterior lobes. Cirrus sac 0.17 to 0.27 by 0.10 to 0.13 millimeter in size, not reaching posteriorly beyond equator of acetabulum. Pars prostatica moderately developed. Cirrus aspinose. Ovary globular or slightly compressed, median, directly preëquatorial. Vitellaria extend from middle of second fourth of body length to posterior end of body. Eggs 78.7 to 85.5 by 54.0 to 60.7 microns in size.

Host.—*Mus norvegicus* Erxleben, 1777.

Location.—Small intestine.

Locality.—Manila, Luzon, Philippine Islands.

Type specimens.—Philippine Bureau of Science parasitological collections, No. 50.

SUMMARY AND CONCLUSIONS

1. In the examination of rats (*Mus norvegicus*) for intestinal and other parasites two echinostome flukes were obtained; namely, *Euparyphium ilocanum, Echinostoma ilocanum* (Garrison, 1908) and *Euparyphium guerreroi* sp. nov.
2. *Euparyphium ilocanum* was first recorded in native Filipinos from Ilocos Sur, Luzon, and subsequently in natives of the neighboring province of Zambales.
3. Its presence in a Manila rat may mean that this human parasite has a wider field of distribution than has formerly been supposed and that rats are a factor in its dissemination.

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ILLUSTRATIONS

ABBREVIATIONS

ac, acetabulum.
cc, cephalic collar.
cs, cirrus sac.
ep, excretory pore.
gp, genital pore.
int, intestine.
lc, Laurer's canal.
oes, œsophagus.
os, oral sucker.
ov, ovary.
ph, pharynx.
pph, prepharynx.
sg, shell gland.
sp, spines.
t, testis.
ut, uterus.
vag, vagina.
vg, vitelline glands.

PLATE 1. EUPARYPHIUM ILOCANUM (GARRISON, 1908)

FIG. 1. Toto mount, ventral view.
2. Anterior end of body, ventral view, showing arrangement of spines on head collar.
3. Body scales.
4. Variations in the shape of the testes.

PLATE 2. EUPARYPHIUM GUERREROI SP. NOV.

FIG. 1. Toto mount, ventral view.
2. Anterior end of body, ventral view, showing arrangement of spines on head collar.
3. Body scales.
4. Variations in the shape of the testes.

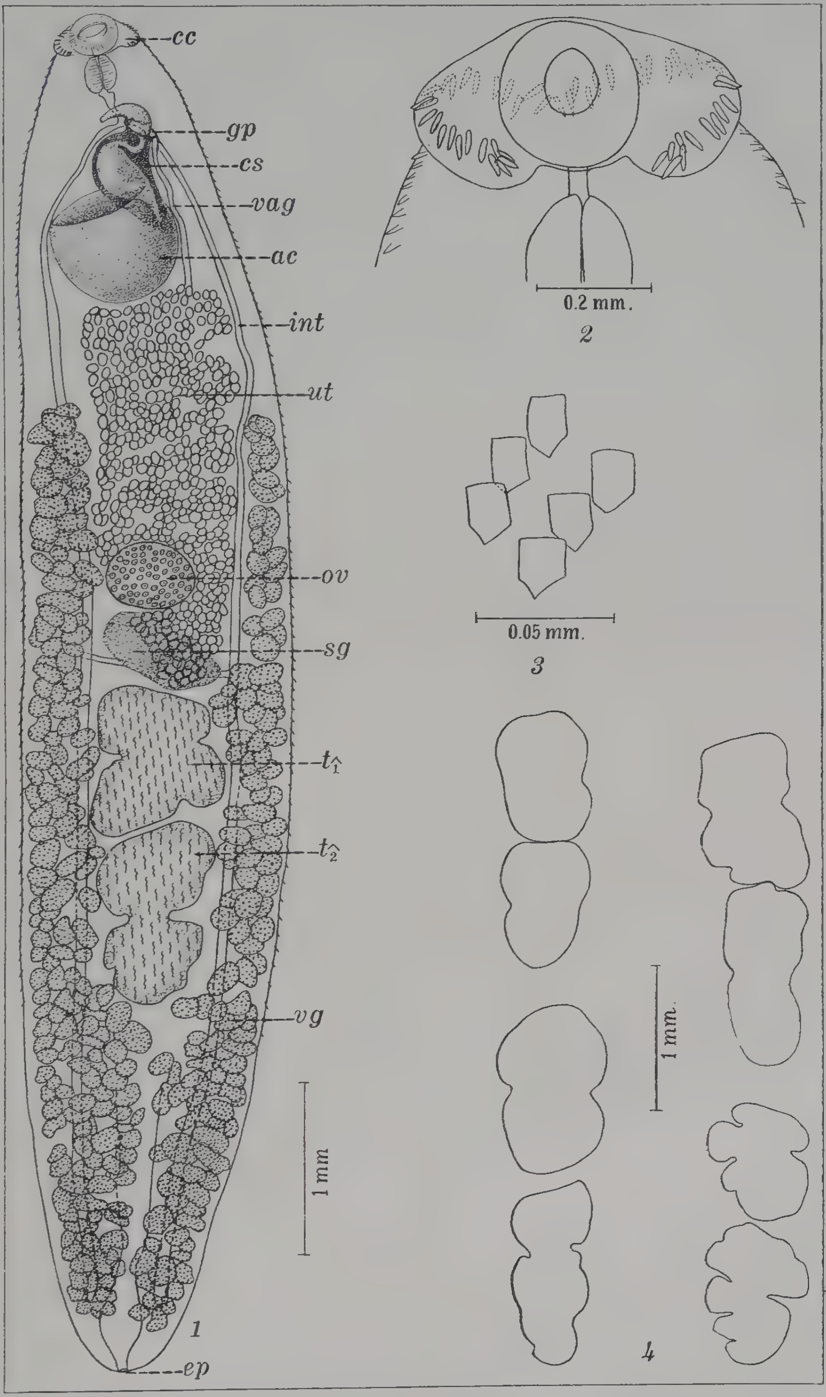
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PLATE 1.

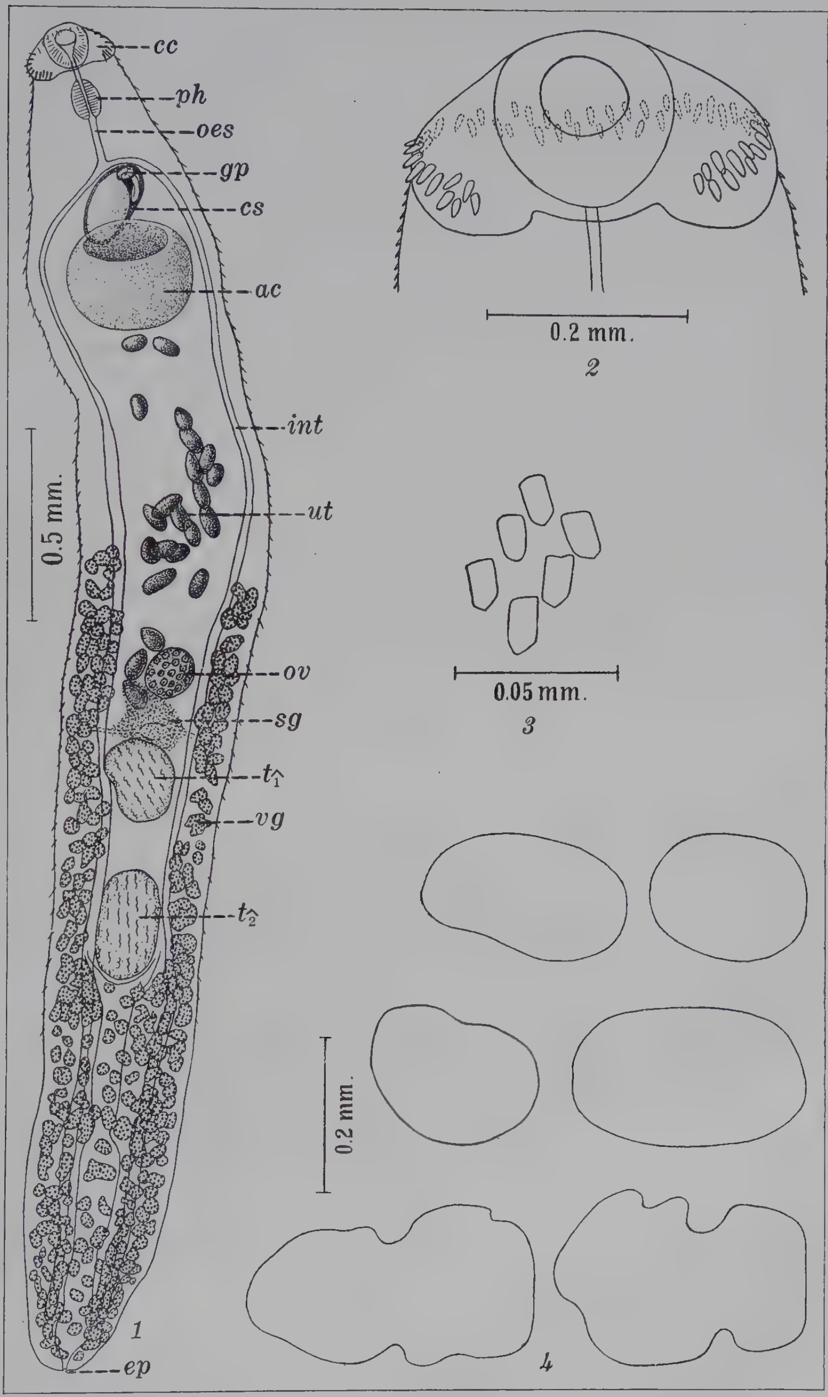
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PLATE 2.

TWO NEW MADREPORARIAN CORALS FROM CALIFORNIA

By Leopoldo A. Faustino
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ONE PLATE

Students of madreporarian corals do not usually regard the west coast of America as particularly productive of corals, although a number of species, both Recent and fossil, have already been reported from the region. As compared with the other coral areas of the world, even in that portion of the coast which is in the tropical zone, namely, the western shores of Central America and Mexico, the reef corals are weakly developed. Specimens of the following two new species of corals were handed me for identification and description in 1924 by Dr. James Perrin Smith when I was at Leland Stanford Junior University, California. The figure of *Dendrophyllia oldroydi* sp. nov. has already appeared.[1]

Family FUNGIIDÆ Dana

Genus SIDERASTREA de Blainville

SIDERASTREA RADCLIFFI sp. nov. Plate 1, fig. 1.

The following is the description of the type, which is a single specimen preserved in the Stanford University palæontological collection.

Corallum incrusting almost the whole surface of a *Turritella* shell. It is not outside the range of probability that the fully grown corallum may be massive or ramose.

Calices irregular in form, subpolygonal and more or less circular. Diameter from 3 to 5 millimeters, when not deformed or prismatic on account of pressure from neighboring calices; depth about 1 millimeter. Synapticulæ show in several places.

Septa in four cycles. The primaries are rather thick and pronounced and reach the calicular center. These are easily distinguished. The secondaries also reach the calicular center, but the tertiaries do not and the quaternaries fuse to the sides of the lower orders. Margins not exsert; within the shallow calices straight or slightly concave upward, within the deep calices descending somewhat abruptly to the calicular center. The septa are trabecular, interrupted, and perforate.

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[1] Stanford University Publications, Geological Sciences, 1: No. 1, pl. 49, fig. 7.

Columella small and slightly developed, sometimes formed by the junction of the ends of the principal septa; occasionally two septa meet in the center and extending across the calice divide it into two, more or less subequal, compartments.

Locality and geologic occurrence.—Camulos Quadrangle, ¾ mile N. E. of Camp near Simi Peak, Ventura County, California, collected by T. D. Radcliff. Martinez formation; lower Eocene.

Remarks.—I have hesitated referring this coral to *Siderastrea* on account of the usually distinct calicular margins, which are not typical of *Siderastrea.* Doctor Vaughan suggested comparison with *Oulastrea* to which it is very closely allied. The genus *Oulastrea,* however, has been reported only as living from the Indian Ocean and it is unlikely that it would suddenly occur in the California Eocene. On the other hand, the genus *Siderastrea* is already represented on the coast by three other species; namely, *S. mendenhalli* Vaughan, *S. californica* Vaughan, and *S. vancouverensis* Vaughan. The Martinez horizon of California Tertiary has yielded but few corals and these are all simple forms. The species described above belongs to the colonial astreiform group, and is the first record of this group to have come from the Eocene formation of the West Coast.

Family EUPSAMMIDÆ Milne Edwards and Haime

Genus DENDROPHYLLIA de Blainville

DENDROPHYLLIA OLDROYDI sp. nov. Plate 1, fig. 2.

Colony high and arborescently branched. Two specimens are in the Stanford University collection; one is 30 centimeters high and 38 centimeters across in one direction, the other is 24 centimeters high with branches not so numerous and with a thick base. The branching is irregular and apparently takes place wherever there is opportunity. Budding takes place at the base of the branches and at the sides. The main stem of the first specimen, which is regarded as the type, is 1 centimeter, and the branches are about the same size. The base of the second specimen is 3 centimeters and the main branches 2 centimeters.

The corallites are subcircular, the largest are 1 centimeter in diameter, and project as much as 7 millimeters above the base. Wall perforate, covered externally with fine subequal costal striations, somewhat scabrous, corresponding to all septa. Corallites not very deep, with a jagged edge.

Septa in four complete cycles, often with narrow rudimentary septa corresponding to the fifth cycle. The form and arrangement of the septa are clearly shown in the figure.

Columella well developed, rather prominent, sometimes occupying about a third of the breadth of the corallite, composed of convoluted and contorted porous plates.

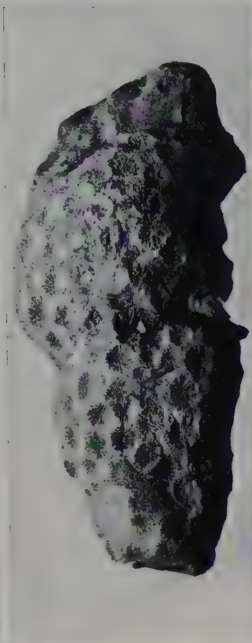
Localities.—Sunken Valley, between San Pedro and Redonda, California, 200 fathoms; deep water off San Diego, California.

ILLUSTRATION

PLATE 1

FIG. 1. *Siderastrea radcliffi* sp. nov., general view of the corallum, natural size; 1*a*, calices of the same, × 4.

2. *Dendrophyllia oldroydi* sp. nov., branch of the type, natural size.



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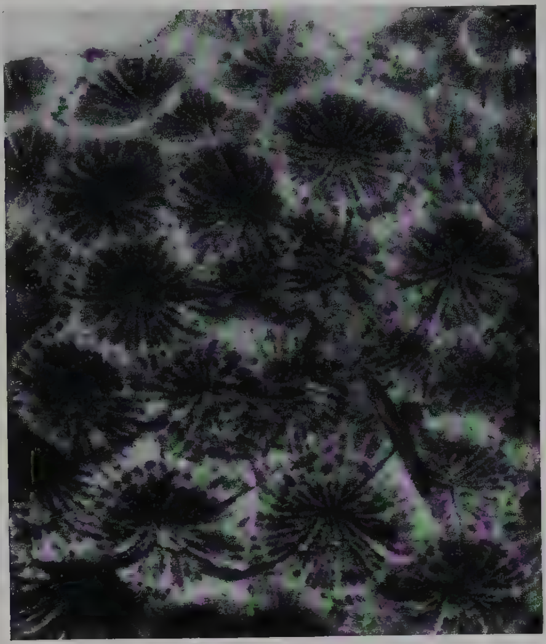

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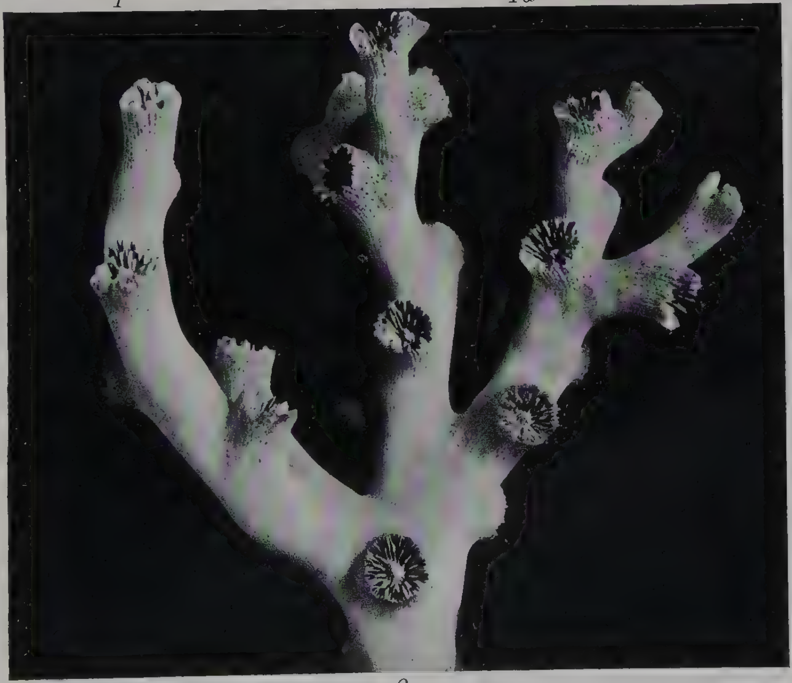

2

PLATE 1.

CORAL REEFS OF THE PHILIPPINE ISLANDS

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THREE PLATES AND THREE TEXT FIGURES

INTRODUCTION

The purpose of the present paper is to present a preliminary report on the coral reefs of the Philippine Islands. It is the first of a series of reports to be made from time to time by the committee on coral reefs of the Philippine Islands, formed at the specific request of Dr. T. Wayland Vaughan in his capacity as chairman of the International Committee of the Pacific Science Congress on the studies of coral reefs and coral-reef phenomena.

The following program of work is proposed by Doctor Vaughan in his circular letter dated July 15, 1927:

I. Associated organisms of the reefs, their ecology, life histories, growth rates, relative importance as constructional agents. Data on temperature, salinity, pH, quantity of plankton, and other data of ecological significance.
II. The marine bottom deposits associated with the reefs. The nature and quantity of the organic matter in the bottom muds. Bacteria associated with the bottom muds specially in lagoon areas.
III. Stratigraphy of the reefs with particular reference to the foundation on which the reefs have been built.
IV. Physiography of the sea-bottom, the shore-line, and the zone interior to the shore-line in each coral reef area.
V. Geologic history of each area in which reefs occur and the relations of the reefs to that history.

LITERATURE AND ACKNOWLEDGMENT

Corals and coral reefs of the Philippine Islands have received the attention of naturalists and scientific men from the time of Darwin to the present day. Darwin's map giving the distribution of the different kinds of coral reefs shows fringing reefs in the Babuyan Islands north of Luzon, on the west coast of

Luzon, southeastern Luzon and northern Samar, southern Masbate, around Bohol, southern and southeastern Mindanao, and around the Sulu Archipelago. Apo Reef, southeast of Mindoro, is marked as an atoll. Dana in his Corals and Coral Islands regarded Sulu Sea as a coral-reef region of some extent, but remarked that for the rest of the Philippine Archipelago coral reefs were little developed. Semper's work on the coral reefs of the Philippines and the Pelew Islands led him to doubt the subsidence theory of Darwin and gave rise to another theory which regards solvent action as the main cause of the formation of barrier reefs. Trinidad Valley near Baguio, Mountain Province, was considered by him an excellent example of an elevated atoll and this conclusion was generally accepted until studies by Dickerson and Smith revealed the fact that it was not an atoll but merely the result of a peculiar erosional development. Arana's Derrotero del Archipiélago Filipino, which is the official Spanish "sailing directions" for the Philippines, notes the presence of the numerous coral reefs. Horsburgh's directions for sailing to and from the East Indies likewise show coral reefs in Philippine waters. Studies of Philippine coral reefs by Davis were embodied in three publications: Fringing Reefs of the Philippine Islands, Subsidence of Reef-encircled Islands, and the Coral Reef Problem. The United States Coast Pilot, Philippine Islands, parts 1 and 2, and the excellent charts of the Philippine Islands published by the United States Coast and Geodetic Survey show the occurrence and the distribution of the coral reefs. The writer's Recent Madreporaria of the Philippine Islands gives an account of the living corals of the Philippine waters. Coral reefs and reef corals of the Philippines have received more or less attention in the different publications touching on the geology and geography of the Philippines.

It is desired to acknowledge with thanks the earnest coöperation of the members of the Philippine Committee on Coral Reefs, more particularly Lieutenant Commander Colbert and the members of the United States Coast and Geodetic Survey stationed in Philippine waters, upon whom I must depend for data not available elsewhere.

LIVING CORAL REEFS OF THE PHILIPPINE ISLANDS

The majority of the coral reefs of the Philippine Islands belong to the class known as fringing reefs, and they rarely exceed 3 miles in width and generally extend less than 1 mile from the shore. Barrier reefs occur rather sparingly and, with the exception of the Bohol Barrier Reef on the northwest coast of Bohol and the Tawitawi Barrier Reef in the Sulu Archipelago, are of small dimensions; the channel between the reef and the shore is generally about 100 to 300 yards wide. Atolls are not rare in the Sulu Archipelago, although it is admitted that they are small and only an extensive cruise in the Sulu Sea or a close examination of large scale charts reveals the presence of a great number of them.

The living coral reefs are less developed in the northern than in the central and the southern Philippines. The islands of the Batan group, the most northern of the Philippine Islands, are surrounded by narrow, disconnected fringes of coral reef. In the Babuyan group, Calayan Island is also fringed by a narrow coral reef. A coral shoal known as Herminia Shoal lies about 2.5 miles off the southwest part of Dalupiri Island, while Camiguin is fringed by a narrow steep-to coral reef. The north coast of Luzon is apparently devoid of coral reefs, as only a narrow coral reef fringes Dialao Point on the northwest coast. The shores of northern Luzon descend precipitously into the sea, and the large Cagayan River discharges its sediments there. The eastern coast of Luzon is imperfectly known.

In the central and the southern Philippines on the other hand the coral reefs are more profusely developed. There are many large and small islands scattered after the manner of most of the archipelagoes in the western Pacific, all more or less embayed. The entire coast line of the Philippine group south of latitude 15° north, with few exceptions, is fronted with coral reefs of varying stages of development. Some are narrow disconnected fringes with or without any living corals; some have parts only slightly submerged and covered with mangroves; others make off from the mainland in a direction tangent to the coast line. The fringing reefs are generally well developed on the headlands and promontories and in small bays or coves or along a straight coast. At the mouths of the larger rivers they are generally dead or covered with sand and with an opening or break across the reef.

The best occurrence of a barrier reef in the Philippines is along the north coast of Bohol. The large Danajon Bank extends northward from Bohol about 40 miles east and west and from 5 to 14 miles north and south. The outer edge of the bank is marked by a chain of steep-to coral reefs, 0.5 to 2 miles wide, with a number of breaks. Depths in the lagoon between the reefs and the mainland are generally from 20 to 25 fathoms. The Northwest Bank, also studded with islets and reefs, is the southwest prolongation of Danajon Bank.

The best development of atolls is found in the Sulu Sea and vicinity. Apo Reef, noted by Darwin, lies southwest of Mindoro and is about 10 miles long in a north-northwest direction and about 6 miles wide. The reef is surrounded by deep water. Pearl Bank, in the Sulu Archipelago, is about 15 miles long northeast and southwest and about 9 miles wide. In the middle is a coral-reef patch, more or less circular in form, about 7 miles in diameter, and bare in patches during low water. Two islets, Taja and Zau, lie on opposite sides of the circular reef. The lagoon in the center is less than 1 fathom deep. Tumindao Atoll is elongated north and south with Tumindao Island forming the eastern rim. It has two lagoons, the North Lagoon and the South Lagoon. The North Lagoon is 8 to 10 fathoms deep with two outlets.

Lying off the southern coast of Zamboanga Peninsula is Santa Cruz Bank, a coral atoll about 2 miles wide and extending 8 miles in a direction parallel to the coast. The channel in the middle of the atoll has a depth of not less than 8 fathoms. The outline of the atoll is marked by shoal patches having depths of 1 foot to 4 fathoms.

In addition to the three main types of coral reefs noted in the preceding paragraphs a fourth type is encountered to which the name "shoal reef" is applied. A shoal reef is here defined as a patch of coral reef below sea level which occurs at a variable distance from the shore and which is surrounded by deep water, say 20 to 40 fathoms and even a greater depth, and which apparently has no direct connection with the fringing reef that borders the land and does not form part of the barrier reef in the locality. The term shoal reef was cited by Molengraaff[1] as having been used by Niermeyer to indicate fringing reefs of broad development on a shelf. The shoal reefs of the Philippines lie in the main on the submerged banks or shelves. Among these may be mentioned the following: Ranger Reef lying a few miles east-southeast of Simara Island, Romblon Province, about 2 fathoms deep and surrounded by deep water. Circe Bank, a shoal reef with a least depth of 3.5 fathoms lies about 10 miles east-northeast of Jintotolo Island, Masbate Prov-

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[1] The Coral Reefs in the East Indian Archipelago, their Distribution and Mode of Development, Fourth Pacific Science Congress 2 (1929).

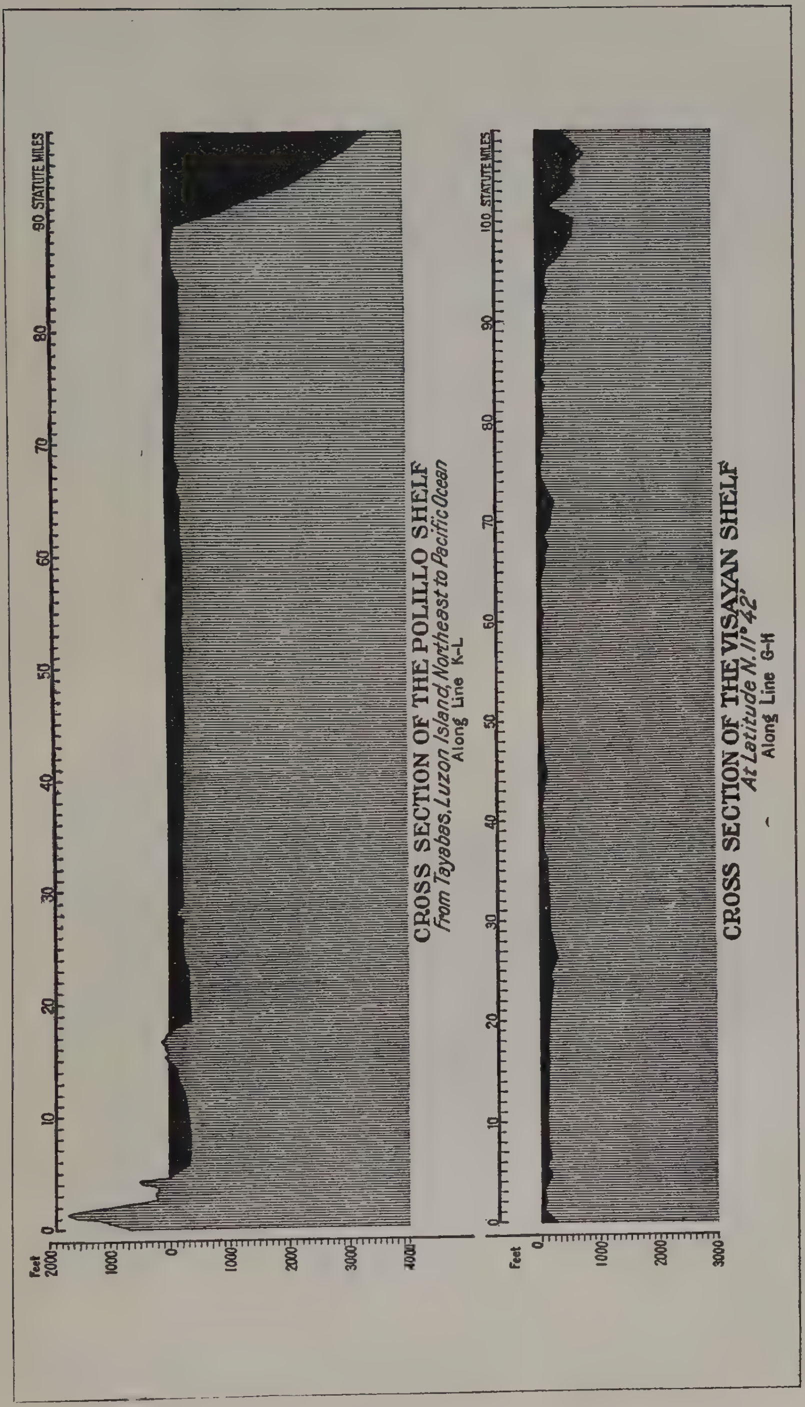


FIG. 1. Cross section of the Polillo shelf and the Visayan shelf.

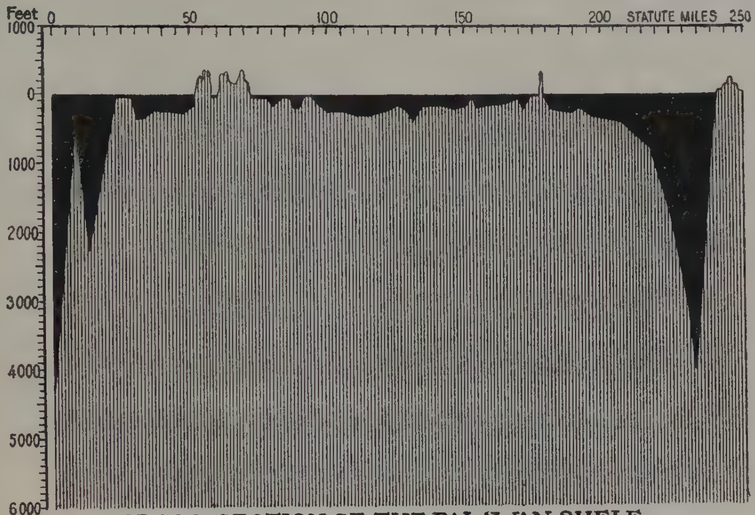
ince. A small coral reef called Sardine Reef, with a least depth of 2.25 fathoms and surrounded by deep water, lies about 1.75 miles west of Ilin Island and 2.5 miles north of Ambulong Island, Mindoro Province. A shoal reef known as Queen of the Sea Bank covers a considerable area in latitude 10° 24.3′ north, longitude 120° 28.5′ east, and has a least depth of 3.5 fathoms. The east coast of Palawan apparently has many coral reefs lying from 10 to 20 miles off the coast. Circe Shoal is a coral bank lying in latitude 8° 26′ north, longitude 117° 56′ east. Balabac Strait, between the China and Sulu Seas, is for the greater part occupied by numerous coral shoals and banks. Fronting the west coast of Palawan is a bank with numerous coral patches and shoals. Among these may be mentioned South Regent Shoal, North Regent Shoal, Antelope Shoal, Scalesby Castle Shoal lying 1.5 miles within the edge of the Palawan shelf, and coral shoals and patches without number from this coral shoal to the parallel of 9° 35′ north, all lying on the edge of the shelf. Crescent Reef lies in latitude 10° 40′ north, longitude 118° 42′ east, within 1.5 miles of the edge of the Palawan shelf and about 22 miles from the nearest land. It is a narrow strip 0.75 mile in length running east-northeast and west-southwest.

SUBMERGED BANKS AND SHELVES

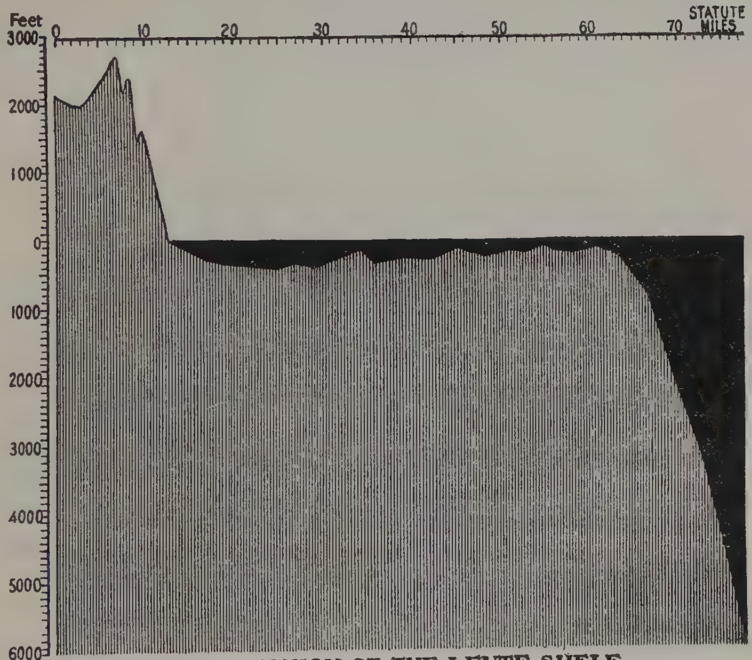
Numerous submerged banks and shelves are found throughout the Philippine Islands. Some of these are of comparatively great dimensions, and are apparently of origin independent of corals. The majority of these, as may be seen by reference to the relief map of the Philippine Islands (Plate 1), are submarine plains lying not more than 50 fathoms below sea level. Among these submerged banks and shelves may be mentioned the Polillo shelf, east of Luzon; the Visayan shelf, between Panay and Masbate; the Palawan shelf, surrounding Palawan Island; the Leyte-Samar shelf, east of Leyte; and the Sulu shelf, west of Mindanao.

The Polillo shelf, on the east of Luzon bordering the Pacific deep, is approximately 150 miles long, along an east-and-west line, and about 50 miles wide. This submarine plain extends from Polillo Island to Catanduanes Island and averages about 25 fathoms below sea level. Numerous islets and coral shoals are scattered on this shelf.

The Visayan shelf is a low-lying submarine platform, located between Panay, Negros, and Masbate Islands and almost in-



CROSS SECTION OF THE PALAWAN SHELF
At Latitude N. 10°55′
Along Line C-D



CROSS SECTION OF THE LEYTE SHELF
At Latitude N. 10°35′
Along Line E-F

Fig. 2. Cross section of the Palawan shelf and the Leyte shelf.

closed by them. The greatest depths on the shelf are on the eastern side and average 25 fathoms, but the greater portion of the shelf is only about 10 to 15 fathoms below sea level.

The Leyte-Samar shelf is located between Samar and Mindanao and is the submarine connection between the two islands. This submarine shelf is long and narrow with a deeper portion in the middle western part. The bank has an average depth of 22 fathoms. Its eastern edge descends precipitously into the Philippine deep.

The Sulu shelf is the submarine prolongation of Mindanao, which connects it with Borneo. This submarine platform maintains an average width of about 50 miles but has a deep channel in the middle. There are depths between 40 to 50 fathoms, but the greater portion of the area averages about 18 fathoms. The islands of the Sulu Archipelago, in part volcanic, in part composed of coral limestone, stand on this bank.

Palawan is a long island extending 250 miles southwest to Borneo and is bordered on all sides by a submarine shelf, which is widest on the north and northeast. The outer edge of the shelf on the west coast runs almost parallel with the shore line, maintaining an average width of 20 miles, and while there are depths of 40 and 50 fathoms the majority of the soundings average about 10 fathoms. The eastern shelf is not so wide, but the platform widens to the north and east. The Calamian Group and the Cuyo Islands stand on the northeastern prolongation of the shelf.

The origin of these submerged banks and shelves presents a very interesting study. Davis pointed out that "the submarine platforms that border some of the islands are best explained as submerged and more or less aggraded reef plains." At present it is believed that these submerged shelves are of Pleistocene origin and that they are due in the main to the shift of ocean level in the tropical seas during Pleistocene time on account of glaciation and deglaciation.. The change in level has been estimated by Daly [2] as between 60 and 70 meters (33 to 38 fathoms). Vaughan [3] pointed out that the period of maximum glaciation was probably not of long duration and the greatest effect of submarine terracing would be expected in somewhat shallower depths, probably between 37 and 55 meters (20 and 30 fathoms). The Pleistocene corals are not so wide-

[2] Proc. Am. Acad. Arts and Sci. 51: 174.

[3] Bull. U. S. Nat. Mus. 103 (1919) 253.

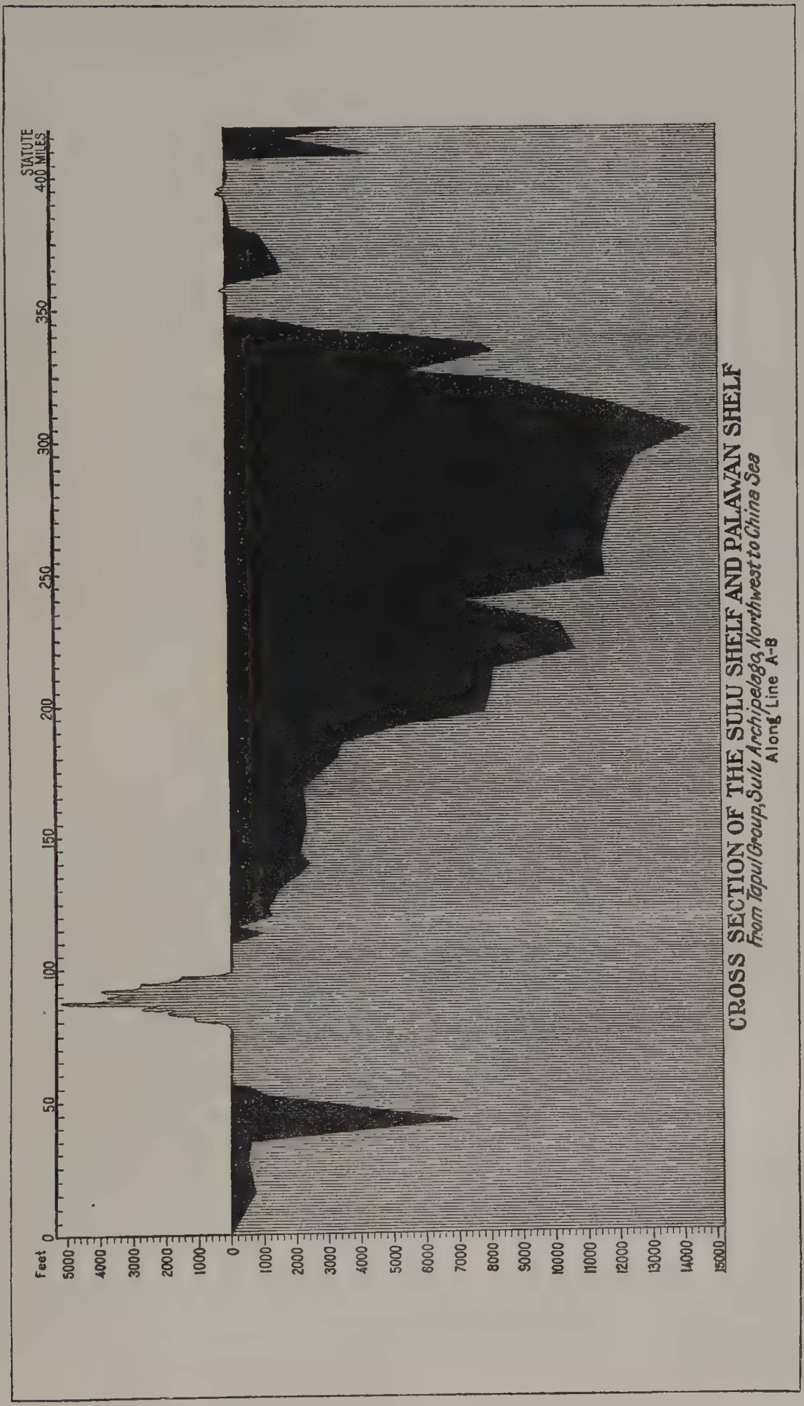


Fig. 3. Cross section of the Sulu shelf and the Palawan shelf.

spread as are those formed in the earlier periods, owing in a large measure to the cold climate that prevailed during Pleistocene time, and the consequent reduction in the rate of formation of the coral reefs. The protection coral reefs afforded the shores being thus lessened, the waves of the sea were able to cut fairly extensive submarine plains.

Reference to the relief map of the Philippine Islands (Plate 1) will show that the submerged banks and shelves correspond in general to the white areas included between the 100-meter isobath and the shore line. Umbgrove in his paper on the amount of the maximum lowering of sea level in the Pleistocene, presented at one of the sessions of the Fourth Pacific Congress in Java (1929), came to the conclusion that the maximum lowering of the sea level in the Pleistocene for the region of the Sunda shelf in the East Indian Archipelago must have amounted to approximately 100 meters. Most of the submarine plains found in the different parts of the Archipelago are less than 100 meters (54 fathoms) below sea level. The difference between this figure and the present depths may be explained by successive uplifts and elevation of the entire Philippine block.

The criteria for recognizing these shelves as submerged submarine plains are the following:

1. Central and southern Philippines show strongly embayed shore lines. Caramoan Peninsula, for example, in the region of the Polillo shelf has a very irregular coast line, bordered by a number of islands, and subaërially eroded valleys are now occupied by the sea. The bedrock of the dredging ground of the Mambulao-Paracale gold district is 58 feet (18 meters) below the present level, and the nature of the bottom gravel indicates that it was deposited by streams flowing above sea level. The upper part of the deposit shows evidences of tidal conditions, and the tide reaches for a long distance inland in some of the streams.

2. Cross sections of these submarine plains show that they are bordered either by steep slopes or by escarpments.

3. The presence of caves in limestone below sea level that are supposed to have been formed by the solvent action of fresh water. Malampaya Sound, in northern Palawan, is a submerged river valley of a former erosion cycle, when the elevation of Palawan above the sea was much greater than it is at present. The underground river in St. Paul Bay, which launches can enter from the sea and navigate for about 4 miles, is an underground channel by which underground water formerly flowed to the sea.

TEMPERATURE AND SALINITY MEASUREMENTS

The United States Coast and Geodetic Survey has taken temperature and salinity measurements in the Sulu Archipelago. The Sulu reefs present excellent examples of coral-reef development in the Philippines, and data on temperature and salinity of the water in the area are of peculiar significance, as they present actual conditions obtaining in a region where coral reefs are known to be well developed. The results of these measurements are given in Table 1.

TABLE 1.—*Temperature and salinity measurements, Sulu Archipelago (United States Coast and Geodetic Survey).*

No.	Latitude north.		Longitude east.		Depth.	Temperature.	Salinity; parts per M.	Sublocality, date, character of bottom, etc.
	°	′	°	′	*Fathoms.*	°C.		
1	4	45.0	119	44.0	12	26.5	35.8	Southwest of Tawitawi Island, G. D. Cowie, 1928; white sand.
2	5	30.0	120	05.35	36	26.7	35.6	North of Tawitawi Island, G. D. Cowie, 1928; white sand.
3	5	32.8	120	10.4	12	26.8	34.9	Do.
4	5	29.5	120	14.1	Surface	28.9	37.6	North of Simaluc Island, L. C. Wilder, 1928; coral and sand.
					30	27.8	38.0	
5	5	29.1	120	14.15	Surface	28.3	35.6	Northeast of Simaluc Island, L. C. Wilder, 1928; coral and sand.
					30.2	26.1	34.8	
6	5	28.7	120	15.05	Surface	27.8	35.4	Do.
					28.0	27.5	35.3	
7	5	30.9	120	15.55	Surface	28.3	33.0	Northeast of Simaluc Island, L. C. Wilder, 1928; fine white sand.
					31.5	26.0	34.0	
8	5	22.3	120	13.4	Surface	28.3	33.0	North of Basbas Island, L. C. Wilder, 1928.
					20.0	28.3	33.0	
9	5	22.9	120	13.9	Surface	28.3	34.8	Do.
					39.5	24.4	33.4	
10	5	23.4	120	16.3	Surface	27.8	34.0	Northwest of Tambagaan Island, L. C. Wilder, 1928; coral sand.
					38.8	24.4	34.1	
11	5	23.9	120	16.45	Surface	28.0	36.3	North of Basbas Island, L. C. Wilder, 1928; coarse white sand.
					38.5	25.2	33.7	
12	5	24.3	120	18.8	Surface	27.8	35.4	North of Tambagaan Island, L. C. Wilder, 1928; fine white sand.
					23.6	27.8	35.4	
13	5	22.8	120	20.85	Surface	27.6	34.5	East of Tambagaan Island, L. C. Wilder, 1928.
					13.0	27.9	34.5	
14	5	25.3	120	21.4	Surface	27.8	34.2	Northeast of Tambagaan Island, L. C. Wilder, 1928; coral sand.
					36.7	22.2	32.4	

TABLE 1.—*Temperature and salinity measurements, Sulu Archipelago (United States Coast and Geodetic Survey)*—Continued.

No.	Latitude north.		Longitude east.		Depth.	Temperature.	Salinity; parts per M.	Sublocality, date, character of bottom, etc.
	°	′	°	′	*Fathoms.*	°*C.*		
15	5	26.2	120	23.0	Surface 26.5	27.2 27.1	32.6 33.5	North of Sigboye Island, L. C. Wilder, 1928; broken coral and sand.
16	5	20.8	120	22.4	Surface 18.0	28.0 28.0	35.5 36.3	Northwest of Pandanan Island, L. C. Wilder, 1928.
17	5	19.8	120	22.1	Surface 20.0	27.8 28.3	35.4 35.6	East-southeast of Tambagaan Island, L. C. Wilder, 1928; coral sand.
18	5	23.2	120	23.8	Surface 18.5	27.5 27.7	34.5 34.5	Southeast of Sigboye Island, L. C. Wilder, 1928; coarse white sand.
19	5	22.7	120	24.8	Surface 15	27.8 28.2	33.4 33.8	South of Sigboye Island, L. C. Wilder, 1928.
20	5	32.1	120	22.5	Surface 30.0	28.3 26.0	33.0 35.5	North of Sigboye Island, L. C. Wilder, 1928.
21	5	31.85	120	22.2	Surface 30.0	27.8 25.0	34.6 33.6	Northwest of Sigboye Island, L. C. Wilder, 1928; coarse white sand.
22	5	33.2	120	25.9	Surface 24.9	28.3 27.4	33.9 32.7	Northwest of Cacataan Island, L. C. Wilder, 1928; coarse white sand.
23	5	32.75	120	25.9	Surface 16.0	27.7 27.2	34.2 34.1	North of Cacataan Island, L. C. Wilder, 1928; coarse white sand.
24	5	33.2	120	27.5	Surface 17.4	27.4 26.6	34.0 34.2	Do.
25	5	36.1	120	31.1	Surface 34.3	28.5 25.0	32.3 32.7	North-northeast of Cacataan Island, L. C. Wilder, 1928; coarse white sand.
26	5	36.7	120	31.65	Surface 34.7	28.7 23.3	34.3 32.3	Northeast of Cacataan Island, L. C. Wilder, 1928; coarse sand.
27	5	27.3	120	33.1	Surface 33.0	28.3 20.6	37.3 33.8	North of Maniacolat Island, L. C. Wilder, 1928; coarse white sand.
28	5	29.0	120	26.5	Surface 31.1	27.8 26.0	34.6 33.1	Southwest of Cacataan Island, L. C. Wilder, 1928; broken coral.
29	5	28.7	120	26.4	Surface 32.0	27.2 27.6	36.0 36.1	Sigboye Pass, L. C. Wilder, 1928; coarse coral.
30	5	28.6	120	28.3	Surface 26.3	27.8 26.8	34.0 34.1	South of Cacataan Island, L. C. Wilder, 1928; coarse white sand.
31	5	25.3	120	27.15	Surface 23.4	27.4 27.6	33.8 34.0	East of Sigboye Island, L. C. Wilder, 1928; coarse white sand.
32	5	24.3	120	27.2	Surface 27.0	27.7 27.2	34.5 35.2	Sigboye Pass, L. C. Wilder, 1928; coarse white sand, broken shells.

TABLE 1.—*Temperature and salinity measurements, Sulu Archipelago (United States Coast and Geodetic Survey)*—Continued.

No.	Latitude north.		Longitude east.		Depth.	Temperature.	Salinity; parts per M.	Sublocality, date, character of bottom, etc.
	°	′	°	′	*Fathoms.*	°*C.*		
33	5	24.5	120	28.1	Surface 22.3	27.8 27.3	35.4 35.2	Southeast of Sigboye Island, L. C. Wilder, 1928; coarse white sand.
34	5	23.7	120	27.6	Surface 26.1	27.8 27.7	34.2 34.3	Sigboye Pass, L. C. Wilder, 1928; broken coral.
35	5	25.2	120	29.8	Surface 16.8	27.7 27.7	35.3 35.4	Sigboye Pass, L. C. Wilder, 1928; red coral.
36	5	26.1	120	31.0	Surface 26.9	27.8 26.0	34.0 34.0	Sigboye Pass, L. C. Wilder, 1928.
37	5	24.6	120	31.1	Surface 32.0	27.2 22.8	33.9 34.6	Sigboye Pass, L. C. Wilder, 1928; coral sand.
38	5	22.85	120	28.45	Surface 27.0	27.8 28.0	34.2 33.8	Sigboye Pass, L. C. Wilder, 1928; coarse white sand.
39	5	22.2	120	27.8	Surface 20.3	27.6 26.7	34.4 34.3	Sigboye Pass, L. C. Wilder, 1928; coral sand.
40	5	21.6	120	28.7	Surface 28.4	27.7 27.2	34.1 34.1	Sigboye Pass, L. C. Wilder, 1928; broken coral.
41	5	18.2	120	30.1	Surface 21.3	27.8 27.7	35.4 35.4	Southeast of Sigboye Island, L. C. Wilder, 1928; broken coral.
42	5	18.3	120	30.8	Surface 19.5	27.6 28.3	34.1 34.9	West of Tagao Island, L. C. Wilder, 1928; broken coral.
43	5	17.0	120	32.5	Surface 14	27.8 27.8	34.0 34.0	Southwest of Tagao Island, L. C. Wilder, 1928.
44	5	19.8	120	33.1	Surface 19.5	27.2 26.8	32.6 33.4	Northwest of Tagao Island, L. C. Wilder, 1928; coral sand.
45	5	22.0	120	33.4	Surface 20.0	27.8 26.4	37.1 36.6	Sigboye Pass, L. C. Wilder, 1928; coarse white sand, red coral.
46	5	23.7	120	33.0	Surface 27.3	27.8 27.6	35.2 34.4	Sigboye Pass, L. C. Wilder, 1928; coarse white sand, broken coral.
47	5	24.2	120	33.1	Surface 31.2	28.2 27.6	34.7 33.6	Sigboye Pass, L. C. Wilder, 1928; broken coral and broken shells.

The temperature measurements are made only at the surface and at depths not exceeding 40 fathoms. The lowest bottom temperature recorded is 20.6° C. at a depth of 33 fathoms, and the average of temperature measurements of depths between 30 and 40 fathoms is 25° C. It will be remembered that these temperatures are much higher than the lower limit recorded by Vaughan,[4] which is an annual minimum not below

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[4] Smithsonian Report for 1917, Washington (1919) 215.

18° C. There is a gradual decrease in temperature from the surface to the bottom for the great majority of records, although eight measurements (Nos. 13, 17, 18, 19, 29, 31, 38, and 42) show slight increases from 0.2° to 0.7° from surface to bottom. Those showing the same or a little higher readings are in a channel communication between Celebes Sea and Sulu Sea. The readings from 32- to 40-fathom depths show a decrease of temperature from 2.8 to 7.7° C. as compared with surface records and an average of 4.3° C.

The salinity measurements do not show any apparent stratification. As a matter of fact, nineteen readings show more salinity at the surface than at the bottom, eighteen other readings show more salinity at the bottom than at the surface, and seven readings have the same salinity at the surface and at the bottom. The highest salinity recorded is 38 parts per thousand at 30 fathoms and the lowest 32.3 at 34.7 fathoms. It will be recalled that the figures given by Vaughan[5] for salinity requirements is between 27 and 38 parts per thousand.

TERTIARY AND PLEISTOCENE REEF CORALS AND CORAL REEFS

The ancient coral reefs of the Philippine Islands have been studied only superficially. Many of these reefs have yielded an abundance of coral species, which are being studied, classified, and named. The coralliferous beds are known in many parts of the Islands, notably along the coasts where they occur as elevated reefs, and also in the interior where they are found in patches and at altitudes up to 1,950 meters (6,318 feet), as on Mount Santo Tomas, near Baguio in Mountain Province. As a matter of fact all provinces and the great majority of the smaller islands have fossil-coral localities. Cebu Island, however, presents the best conditions for the study of reef corals and coral reefs.

SUMMARY AND CONCLUSIONS

The general results of the foregoing preliminary survey may be summarized as follows:

1. Living coral reefs are best developed in the central and southern part of the Philippines, more particularly in the region of the submerged banks and shelfs; namely, on the Palawan shelf, the Sulu shelf, the Visayan shelf, and the Polillo shelf.

2. The majority of the fringing reefs are limited in breadth, rarely exceeding 3 miles and averaging less than 1 mile wide.

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[5] Loc. cit.

This condition is taken as a proof of the apparent instability of the Philippine land mass so that only in very few places has the coast line remained stationary sufficiently long to permit the fringing reef that has developed to increase in breadth by outward growth.

3. Coral reefs have grown and developed whenever and whereever conditions are satisfactory for the growth and development of corals. In other words depth, temperature, ocean currents, salinity, and other conditions have been responsible for the location of coral reefs rather than the presence of any land area. The form developed by the growing reefs is determined principally by the ocean currents and the direction of the wind so that the generally accepted classification of fringing, barrier, and atoll reefs should not be taken with evolutionary significance but rather convenient forms as means of reference. In addition to the reefs fringing the larger and smaller islands there are very many coral-reef patches not rising above the sea level, some distance from the land, surrounded by deeper waters and apparently without connection with the neighboring reefs. To this class of reefs the term shoal reef has been applied. The fringing reefs do not have the same extension from the shore line, but in a great many cases project for some distance beyond the end or point of the island, and in some cases do not follow the shore line but form a spit or take a direction tangent to the coast.

4. Tertiary and Pleistocene coral reefs are widely scattered throughout the Archipelago and their geologic history can be ascertained.

ILLUSTRATIONS

PLATE 1

Map of the Philippine Islands showing land in black and submarine shelves in hatchings. Based upon Coast and Geodetic Survey map No. 4200.

PLATE 2

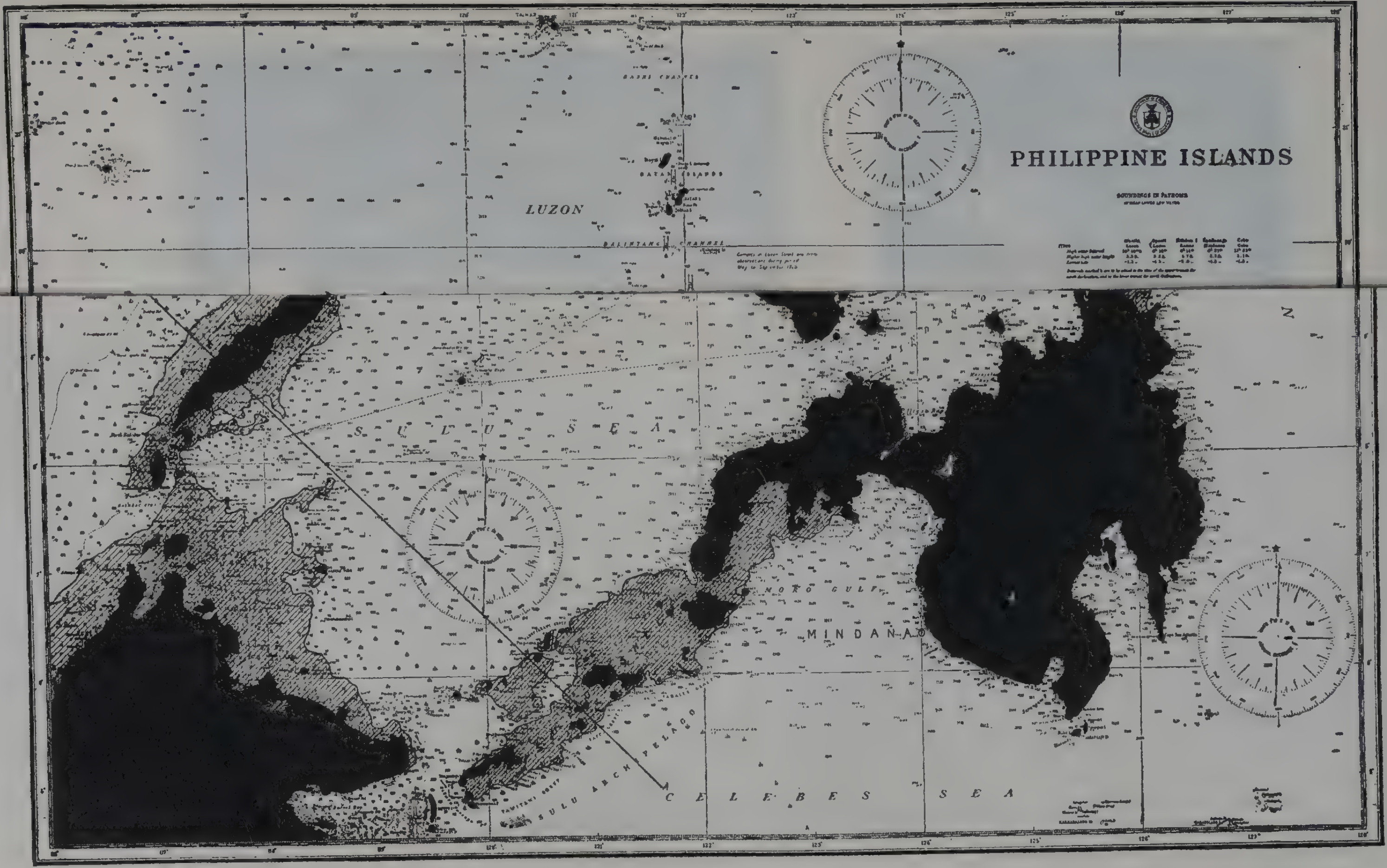
FIG. 1. Coralliferous limestone cliff, Cebu.
2. Underground river in St. Paul Bay, Palawan.

PLATE 3

FIG. 1. Maeander Reef, Sulu Sea.
2. Fringing reef, west coast of Luzon, partly exposed at low tide.
3. Coast near Santa, Ilocos Sur, west coast of Luzon, showing raised coral reefs on Abra fault scarp. (Photograph by Bailey Willis.)

TEXT FIGURES

FIG. 1. Cross section of the Polillo shelf.
2. Cross section of the Visayan shelf.
3. Cross section of the Leyte-Samar shelf.
4. Cross section of the Palawan shelf.
5. Cross section of the Palawan shelf and the Sulu shelf.


PHILIPPINE ISLANDS
SOUNDINGS IN FATHOMS
BASHI CHANNEL
BATAN ISLANDS
LUZON
BALINTANG CHANNEL
SULU SEA
MORO GULF
MINDANAO
SULU ARCHIPELAGO
CELEBES SEA
N

The Philippine Islands showing land in black and submarine shelves in hatchings. Based upon coast and Geodetic Survey map No. 4200.

PLATE 1.

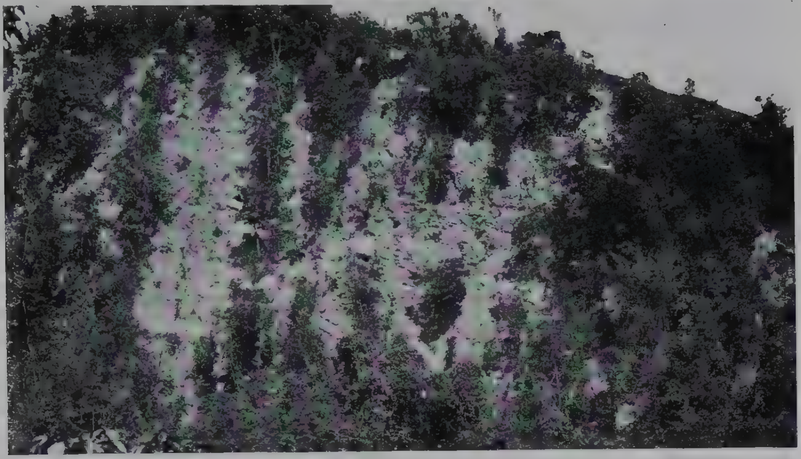


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PLATE 2.

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2

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PLATE 3.

A SURVEY OF MONGREL DOGS OF MANILA, PHILIPPINE ISLANDS, FOR HELMINTHIC AND BLOOD PROTOZOAN PARASITES [1]

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Majors, Medical Corps, United States Army

and

Francois H. K. Reynolds
Captain, Veterinary Corps, United States Army

The present report of a survey of mongrel dogs at large in the City of Manila, Philippine Islands, for helminthic and blood protozoan parasites is based in the first instance on autopsy examinations of fifty, and in the second on the examination of thin blood smears from one hundred dogs. Aside from the recording of the index of parasitization of dogs in the Philippines, this report may be of interest in showing that the helminthic rate of infestation of dogs in Manila is not greater than would be expected in cities of similar size of temperate climates.

Table 1.—*Result of the survey for helminths present in fifty dogs.*

Parasite.	Dogs positive.	Number of helminths present.		
		Maximum.	Minimum.	Average.
	P. ct.			
Ancylostoma caninum	100	169	2	32.34
Dipylidium caninum	34	20	1	10.29
Wuchereria immitis	10	4	2	2.20
Toxacaris canis	4	6	2	4.00

Only one species of hookworm was found; namely, *Ancylostoma caninum*. This species was present in all the dogs examined. The average number present per dog was 32.34. Only two, or 4 per cent, of the dogs harbored more than one hundred hookworms, while in 40 per cent of the animals there were less than fifty per dog.

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[1] From the United States Army Medical Department Research Board, Bureau of Science, Manila, Philippine Islands.

The filaria were found in the right side of the heart, and in each animal the peripheral blood contained the larval forms.

In the examination of one hundred dogs for blood parasites, 4 per cent were positive for *Babesia canis*. All of these positive dogs were approximately half grown. Smears from the older dogs were negative.

With one exception, all the *Babesia* infections were light; the parasites numbering approximately three to five per drop of blood. In one instance a leukemia coexisted with a heavy infection; the total white blood cells present in this instance was 340,000 per cubic millimeter and the differential count was as follows:

	Per cent.	Per cent.
Polymorphonuclears		33.4
Young neutrophiles	9.2	
"Staff" forms	19.8	
"Segmented" forms	4.2	
Eosinophiles	0.2	
Basophiles	0.0	
Monocytes (?) and lymphoblasts		49.0
Lymphocytes		17.6

Of the lymphoblasts, eight were of the lobulated or "Reider" type. "Inclusion bodies" (nucleoli) were seen in twelve lymphoblasts (?) and one lymphocyte.

Only one species of tick was found infesting the dogs positive for *B. canis;* namely, *Rhipicephalus sanguineus*. Transmission experiments of *B. canis* were conducted with *R. sanguineus* removed from infected dogs as follows:

EXPERIMENT 1

March 5, 1930.—Four nursing puppies, 3 weeks old, and the mother were used. Preliminary smears were negative for *B. canis,* but all dogs were infested with *R. sanguineus*. Ticks collected February 2, 1930, from positive dogs 40 and 183, were placed on all. March 11 one of the puppies was positive and died as a result of the infection March 12. The infection in this instance was heavy, nearly every cell being parasitized and some cells contained multiple pairs. Smears from the three other puppies were negative, but March 19 a scant infection was noted in the mother dog, but this animal never became ill.

EXPERIMENT 2

March 20, 1930.—Ticks (*R. sanguineus*) were removed from the mother dog noted in experiment 1 and placed on half-grown dog 2. March 25 the dog was very sick, all food was refused, and it was difficult for her to stand. Smears were positive for *B. canis* on this date; the degree of infection was regarded as moderate. The blood remained positive for a period of seven days, after which the animal recovered.

As it is already well known that *Rhipicephalus sanguineus* is the common transmitting agent of *B. canis* in other parts of the world, the experiments reported above were planned merely to obtain evidence that this tick is probably the common vector of *B. canis* in the Philippines. The results of experiments 1 and 2 indicate that the incubation period of tick-transmitted piroplasmosis in dogs is about five days, and that the virulence of the infection decreases with the age of the definitive host. This is in accordance with well-known facts.

SUMMARY

1. At autopsy on fifty dogs obtained in the City of Manila, it was found that with the exception of filaria, the percentage infested with and the average number of helminths present per dog were not greater than would be expected in stray dogs in cities of temperate climate.
2. In a survey of one hundred dogs for blood protozoan parasites, 4 per cent were positive for *Babesia canis*. This parasite was transmitted experimentally in three instances from dogs found infected in nature to healthy animals, by *Rhipicephalus sanguineus*.
3. The results of the transmission experiments of *Babesia canis* by *Rhipicephalus sanguineus* indicate that the incubation period of piroplasmosis in dogs is about five days and that the infection may be severe in young, moderate in half-grown, and mild in older dogs.

THYROID GLAND OF THE CARABAO

By Manuel D. Sumulong

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TWO PLATES AND TWO TEXT FIGURES

The thyroid gland, peculiar to mammals (1910), has engaged the attention of many investigators, among whom the following may be mentioned: Rumph and Smith (1926), Kull (1926), Moody (1910), Norris (1916), Cowdry (1922), Bensley (1916), Jackson (1916), Major (1909), Hammet (1927), Dye and Manghan (1929), and Williamson (1926). A survey of their respective work, however, has shown that their attention has been focused principally upon the histogenesis, morphogenesis, and physiology of the gland, and upon the rôle it plays in the growth of various organs. It has been noted also that literature on hand bearing on the anatomy of the thyroid gland of various domestic animals fails to show any previous description of this gland in the carabao. The study here reported was undertaken in an attempt to gain some information on the subject; it is believed that this will be of interest to students of comparative anatomy.

The following account is based upon the data obtained from the dissection of the ventral part of the necks of ten carabaos of different ages and microscopic examination of the thyroid gland found in each animal.

COMPARATIVE ANATOMY

According to Sisson (1917) the thyroid gland of the ox is pale in color in the adult and dark red in the calf, and it is softer in texture than in the horse. The lateral lobes average about 8 centimeters in length and are irregularly triangular in outline and more extensive and flatter than in the horse. They are situated on each side of the trachea near to or in contact with the larynx; in addition to the trachea they are also related deeply to the œsophagus and the cricopharyngeal muscle to a varying extent. The isthmus is constantly present and glandular.

In the sheep (Sisson) the thyroid gland is dark red. The lateral lobes, which are long elliptical in outline, lie on each side of the neck over the first six or seven tracheal rings. Each lobe is from 5 to 6 centimeters in length and about 1.5 centimeters in height. The isthmus is also constantly present and glandular.

Vaughan (1909), Bradley (1896), and M'Fadyean (1910) describe the thyroid gland of the horse as brownish red. The lateral lobes are ovoid in outline and are joined by a narrow isthmus; the latter is sometimes reduced to a small strand of fibrous connective tissue. According to Vaughan and Bradley the lateral lobes are related to the upper part of the trachea on each side, resting on the first two tracheal rings, but M'Fadyean states that they lie over the first four tracheal rings.

Caylor and Schlotthauer (1927) made a very comprehensive and thorough study of the anatomy of the thyroid gland of swine, and according to these investigators it is situated on the ventral surface of the trachea in the median line just anterior to the thoracic inlet, in the angle formed by the carotid arteries and the jugular veins. The two lateral lobes are fused in the median line so as to form a single organ. It is deep red, and the average dimensions in adult swine are 6 by 4 by 3 centimeters.

In the dog (1917), the lateral lobes of the thyroid gland are long and narrow, and have a flattened, ellipsoidal form. They are situated on the lateral surface of the trachea near the larynx, extending along the first six or seven tracheal rings. The isthmus is inconstant and variable, being present always in large, but not in small species.

GROSS ANATOMY OF THE THYROID GLAND OF CARABAO

COLOR, FORM, SIZE, AND LOCATION

Carabao calf, about 2 years old.—The thyroid gland of this animal was brownish red, and the lateral lobes, which were connected by a rather wide glandular isthmus—double in its middle part—were asymmetrical in position, size, and form. The left lateral lobe was somewhat compressed laterally, having a convex superficial surface and slightly concave deep surface. It was more or less elliptical in outline, and its anterior end was very much thicker than the posterior one. The latter was further characterized by the presence of a tail-like projection. This lobe was situated along the dorsolateral part of the first four tracheal rings, its anterior end coming in contact with the cricothyroideal muscle. It was 3.8 centimeters in length and 1.7 centimeters in height. The right lateral lobe was more or less bean-shaped and much smaller than its fellow, being 1.7 centimeters in length and 1.5 centimeters in height. It was observed to be lying along the first two tracheal rings only.

Carabao bull, about 3 years old.—The thyroid gland in this animal differs from that of the preceding one only in size and form of its lobes. The left lobe was more or less elliptical in outline and 4 centimeters in length by 2 centimeters in height, while the right lobe was ovoid in form and 1.9 centimeters in length by 1.7 centimeters in height.

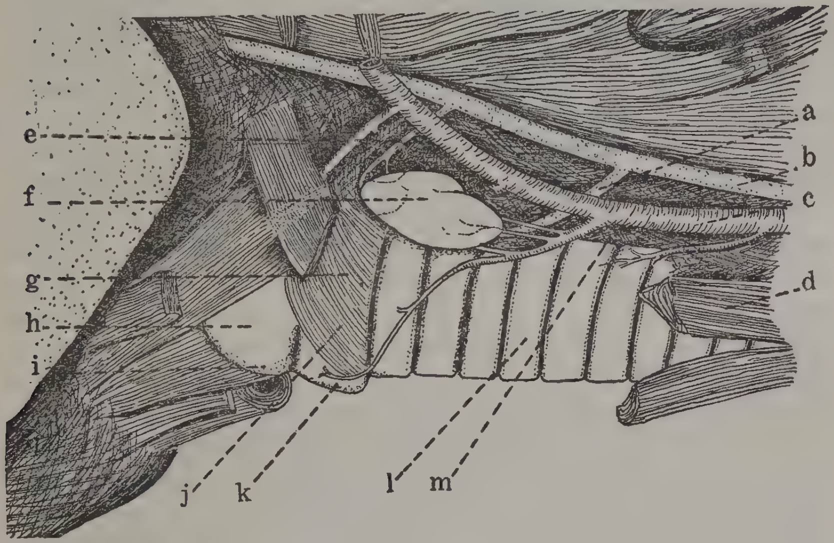

FIG. 1. Left side of the neck (dissected) of an adult carabao, showing the form, location, and blood supplies of the left lateral lobe of the thyroid gland. *a*, Posterior thyroid artery; *b*, common carotid artery; *c*, internal jugular vein; *d*, sternothyrohyoideus; *e*, thyrolaryngeal artery; *f*, left lateral lobe of the thyroid; *g*, *j*, cricothyroideus; *h*, thyroid cartilage; *i*, laryngeal prominence; *k*, arch of cricoid cartilage; *l*, tracheal cartilage; *m*, œsophagus.

Carabao bull, about 4 years old.—The thyroid gland here was likewise brownish red. The left lateral lobe was in the form of a hen's egg; it was over the first three tracheal rings and the posterior half of the cricoid cartilage. It measured 4.2 centimeters in length and 2.7 centimeters in its greatest height. The broader posterior end or pole was much thicker than the anterior one. The right lateral lobe was more or less ovoid, and it lay over the first three tracheal rings only. This lobe was shorter than the left one, its length being 3.5 centimeters only; its greatest height, however, was 2.7 centimeters. The isthmus was broad and distinctly glandular, and it was attached to the middle of the ventral border of the right lateral lobe and to the ventral border of the left lobe a short distance behind its middle.

Carabao bull, about 7 years old.—In this carabao the thyroid gland was no longer brownish red but dark red. The lateral lobes were observed to be asymmetrical both in size and form. The left lobe was more or less ellipsoidal in outline; it was situated on the dorsolateral aspect of the trachea, lying along the first three tracheal rings and the cricoid cartilage. It measured 4.5 centimeters in length and 2.8 centimeters in height. The right lobe was more or less triangular in outline and much flatter than its fellow. It lay along the first three tracheal rings and measured 3.7 centimeters in length and 3 centimeters in height. Its ventral angle was connected to the middle of the ventral border of the opposite lobe by a narrow isthmus which was very much lighter in color than the lobes.

Carabao cow, about 9 years old.—The gland in this animal was also dark red. The left lateral lobe was rather elongated, having a rounded anterior end and a blunt-pointed posterior end. It extended along the first four tracheal rings and the cricoid cartilage. Its length was 5.2 centimeters, and its greatest height was 2.3 centimeters. The right lateral lobe was very much flattened and irregularly triangular in outline, having its base directed dorsally; its ventral angle was prolonged to a short narrow band which was apparently the only glandular part of the isthmus, the continuation of the latter to the opposite lobe being represented only by fibrous connective-tissue strands. The anterior and posterior angles are rounded; the lateral surface was smooth and slightly convex while the medial surface was irregularly concave. This lobe was in contact with the first two tracheal rings and the posterior half of the cricoid cartilage. Its base was 3.8 centimeters in length, and its height was 4.2 centimeters.

Carabao bull, about 10 years old.—Except for a little difference in the size of the lateral lobes, the thyroid gland in this animal was essentially identical to that of the preceding one.

Carabao cow, about 12 years old.—The thyroid gland in this animal was dark red. The left lateral lobe, which was ellipsoidal in outline, extended over the first three tracheal rings

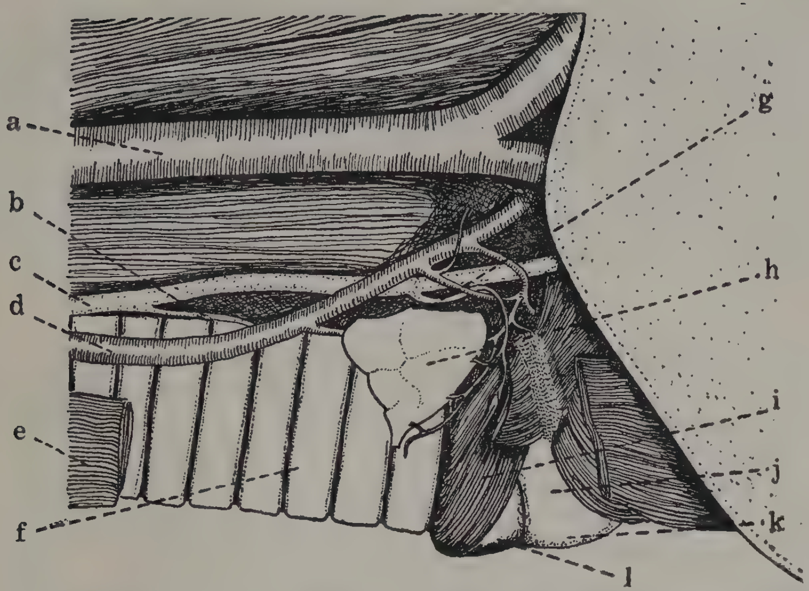

FIG. 2. Right side of the neck (dissected) of an adult carabao, showing the form, location, and blood supplies of the right lateral lobe of the thyroid gland. *a,* External jugular vein; *b,* posterior thyroid artery; *c,* common carotid artery; *d,* internal jugular vein; *e,* sternothyrohyoideus; *f,* tracheal cartilage; *g,* thyrolaryngeal artery; *h,* right lateral lobe of the thyroid gland; *i,* cricothyroideus; *j,* thyroid cartilage; *k,* laryngeal prominence; *l,* arch of cricoid cartilage.

and the cricoid cartilage. It was 4.8 centimeters in length and 3 centimeters in height. The right lateral lobe was more or less triangular in outline and it lay along the first three tracheal rings. It measured 3.7 centimeters in length and 3.9 centimeters in height. The isthmus was represented only by a narrow strand of fibrous connective tissue connecting the ventral angle of the right lobe and the ventral border of the left lobe a short distance behind its middle.

Castrated male carabao, about 15 years old.—The thyroid gland here was also dark red. The left lateral lobe was elliptical and extended over the first three tracheal rings and the cricoid cartilage. It was 4.8 centimeters in length and 2.8 centimeters in height. The right lateral lobe was also flattened and irregularly triangular. This lobe was in contact with the first three tracheal rings and part of the cricoid cartilage. It measured 3.8 centimeters in length and 4.2 centimeters in height. The isthmus could not be distinguished.

Carabao cow, about 18 years old.—In this animal the left and right lateral lobes were practically the same in outline and location as those in the preceding animal. The left lateral lobe was 4.8 centimeters in length and 3.2 centimeters in height, while the right one was 5 centimeters in length and 4.6 centimeters in height. The gland was likewise dark red. The isthmus was totally absent.

Castrated male carabao, about 20 years old.—The thyroid gland here was almost similar in all respects to that of the 18-year old carabao cow.

RELATIONS

The thyroid gland in all the carabaos studied, irrespective of sex and age, was observed to possess a rather dense connective-tissue capsule by means of which its lateral lobes were loosely attached to the trachea. It has been noticed also that the thyroid gland in this animal has practically the same relations as in the ox. Superficially it is related to the external thyrohyoideus, omohyoideus, part of the parotid gland internal jugular vein, vagus and sympathetic nerves, and deeply to the dorsolateral aspect of the anterior part of the trachea, cricothyroideal muscle, and the œsophagus. Sometimes the right lateral lobe of the thyroid gland does not come in contact with the œsophagus.

BLOOD SUPPLY

Summing up the observations on all the animals dissected, the blood supply of the thyroid gland in the carabao may be described as follows: Each lateral lobe of the thyroid gland of the carabao receives blood from two collateral branches of the common carotid artery, the thyrolaryngeal artery and a branch which corresponds to the posterior thyroid artery in the horse. The thyrolaryngeal artery on either side is very short and not infrequently smaller than the corresponding posterior thyroid artery. It divides into two principal branches; namely, the laryngeal artery and the thyroid artery. The laryngeal artery gives rise to small branches which ramify the intrinsic muscles of the larynx. On the right side the thyroid artery enters the gland along the anterior border and at the anterior angle by means of numerous small branches. On the left side the artery enters the gland at the anterior pole. The posterior thyroid artery is variable in size and origin in different subjects and sometimes also in the same individual. It is generally larger, however, than the thyrolaryngeal artery. On the right side it may arise as far back as the level of the ninth tracheal ring, and on reaching the gland it breaks into three or more primary branches which penetrate the gland at the posterior end or angle. The left posterior thyroid artery is usually larger than its fellow and it arises farther in front; it enters the posterior end of the left lateral lobe by two or three branches. The corresponding thyroid veins are large and empty into the internal jugular vein.

MICROSCOPIC ANATOMY

The microscopic appearance of the thyroid gland of the carabao resembles very closely that in other animals. The follicles vary greatly in size, form, and the diameter of the lumen. Sections of the majority of them, however, are round or ovoid in form; those that are far apart from each other, separated by much interfollicular connective tissue, are generally spherical, whereas those that are closely packed are pentagonal or hexagonal in outline. The follicles near the capsule and the primary trabeculæ are generally smaller and less distended, and the colloid they contain is much more vacuolated than in the interior. In the interior of the gland are found a few exceptionally large follicles containing colloid substance in which red blood corpuscles are embedded. The small follicles are lined by simple epithelium whose cells are typically cuboidal; the large ones, especially those that are distended with colloid, the epithelial cells are almost pavement in type. The epithelial cells lining the empty follicles are more or less short columnar in type.

The interfollicular tissue is relatively greater in amount in areas where the majority of the follicles are small. In the interfollicular tissue or stroma are seen epithelial cells which are irregularly scattered; blood capillaries are likewise observed. Lymphocytes are also found in the stroma in small numbers; they are more numerous at the periphery of the gland. In the interior of the lobe are recognized areas with few small follicles between which the stroma is closely packed with epithelial cells. The blood capillaries in the interfollicular tissue between adjacent large follicles are hardly, if ever, distinguishable. There appear no marked differences between the microscopic picture of the thyroid gland from a young animal and that from a full-grown or old one, the size and the number of follicles as well as the proportion between the cellular elements and the colloid substance being practically identical.

SUMMARY AND CONCLUSIONS

1. The present account is the result of an investigation on the gross and microscopic anatomy of the thyroid gland of ten carabaos which, so far as I am aware, has not yet been reported.

2. The thyroid gland of the carabao is bilobed as in the ox, sheep, horse, and goat, and the lateral lobes are generally brownish red in young animals but dark red in adults.

3. The left and right lateral lobes of the thyroid gland in young animals vary in shape, but in full-grown individuals, irrespective of sex, the left lobe is generally elliptical or ellipsoidal in outline and the right one irregularly triangular.

4. In full-grown animals the left lobe measures from 4.5 to 5.2 centimeters in length and 2.3 to 3.2 centimeters in its greatest height, and the right lobe varies from 3.7 to 4 centimeters in length and from 3 to 4.6 centimeters in height. Invariably, both in the young and adult animals, the right lateral lobe is flatter and less voluminous than the left one.

5. The isthmus is constantly present and glandular in young subjects, but in adults it may be represented only by fibrous connective-tissue strands; in old subjects it is generally absent.

6. In the adult carabao the lateral lobes of the thyroid gland are situated along the dorsolateral aspect of the trachea, extending over the first three tracheal rings and the posterior half of the cricoid cartilage. Not infrequently the right lateral lobe does not extend so far forward as to lie over the cricoid cartilage. In young animals the position of this gland varies a little.

7. The thyroid gland in the carabao has practically the same relations as in the ox; the right lateral lobe, however, is not always in contact with the œsophagus.

8. The gland receives its blood supply from the thyrolaryngeal artery and from another collateral branch of the common carotid artery which corresponds to the posterior thyroid artery in the horse. The corresponding thyroid veins are radicles of the internal jugular.

9. The microscopic anatomy of the thyroid gland of the carabao does not materially differ from that of other animals. The microscopic appearance of the gland from the young animal presents no striking difference from that of the adult.

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ILLUSTRATIONS

PLATE 1

Photograph of the ventral aspect of the neck (partly dissected) of a carabao about 10 years old, showing the location of the lateral lobes of the thyroid gland. *a*, *a'*, Lateral lobes of the thyroid gland; *b*, internal jugular vein; *c*, common carotid artery; *d*, external jugular vein; *e*, sternothyrohyoideus; *f*, omohyoideus. (Photograph by the College of Agriculture.)

PLATE 2

Microphotograph of a section through the lateral lobe of the thyroid gland of a carabao 7 years old. *a*, Colloid substance with red blood corpuscles in one of the exceptionally large follicles in the interior of the lateral lobe. (Photograph by the Bureau of Science.)

TEXT FIGURES

FIG. 1. Sketch of the left side of the neck (dissected) of an adult carabao, showing the form, location, and blood supplies of the left lateral lobe of the thyroid gland. *a*, Posterior thyroid artery; *b*, common carotid artery; *e*, internal jugular vein; *d*, sternothyrohyoideus; *e*, thyrolaryngeal artery; *f*, left lateral lobe of the thyroid; *g*, *j*, cricothyroideus; *h*, thyroid cartilage; *i*, laryngeal prominence; *k*, arch of cricoid cartilage; *l*, tracheal cartilage; *m*, œsophagus.

2. Sketch of the right side of the neck (dissected) of an adult carabao, showing the form, location, and blood supplies of the right lateral lobe of the thyroid gland. *a*, External jugular vein; *b*, posterior thyroid artery; *c*, common carotid artery; *d*, internal jugular vein; *e*, sternothyrohyoideus; *f*, tracheal cartilage; *g*, thyrolaryngeal artery; *h*, right lateral lobe of the thyroid gland; *i*, cricothyroideus; *j*, thyroid cartilage; *k*, laryngeal prominence; *l*, arch of cricoid cartilage.

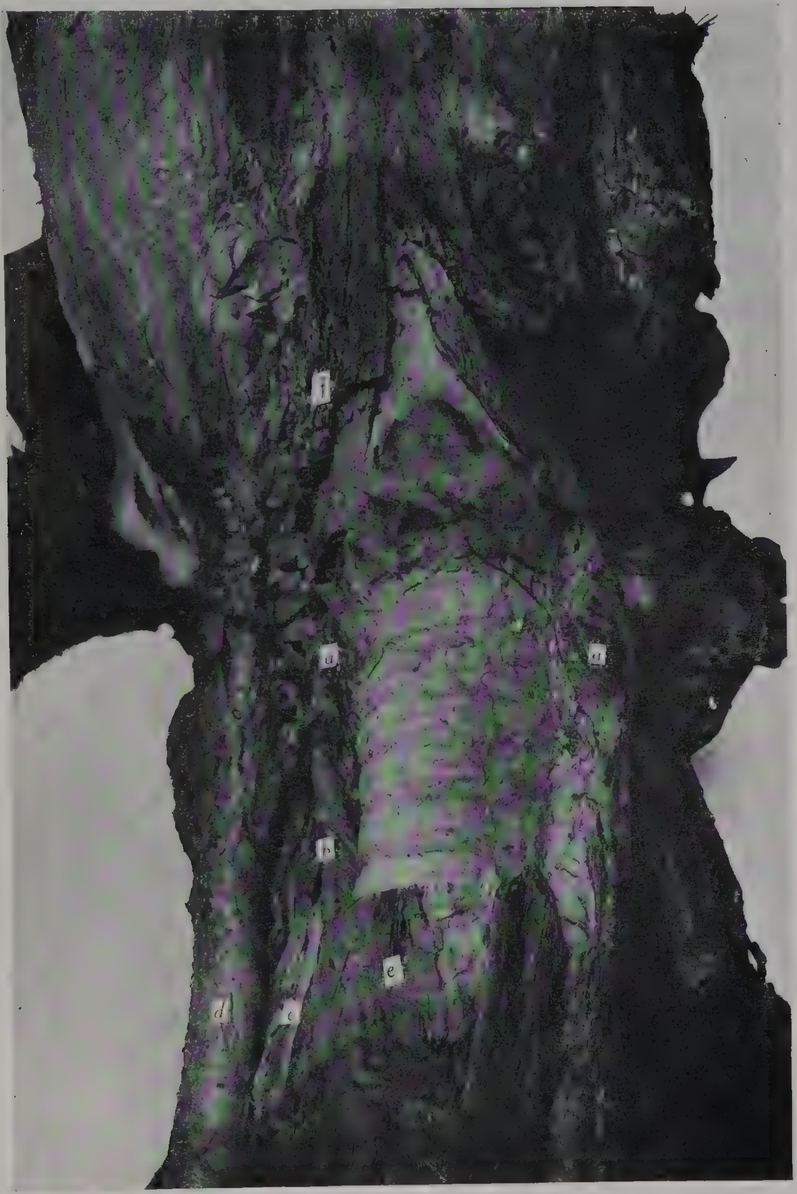

PLATE 1. VENTRAL ASPECT OF THE NECK (PARTLY DISSECTED) OF A CARABAO ABOUT 10 YEARS OLD, SHOWING THE LOCATION OF THE LATERAL LOBES OF THE THYROID GLAND.

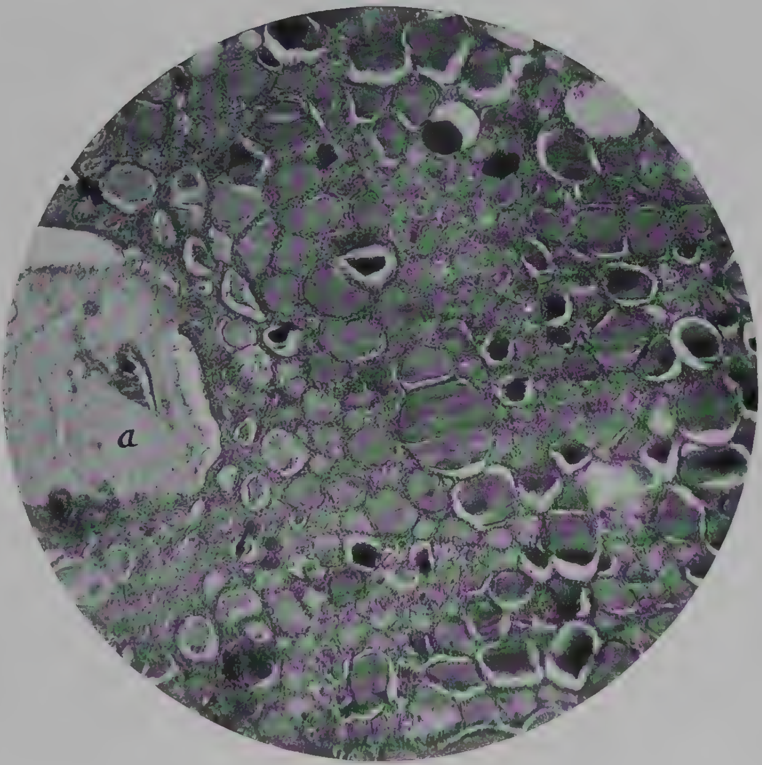

PLATE 2. SECTION THROUGH THE LATERAL LOBE OF THE THYROID GLAND OF A CARABAO 7 YEARS OLD.

VITAL-CAPACITY STUDIES AMONG FILIPINOS

VITAL-CAPACITY STANDARDS FOR MEN FROM 15 TO 30 YEARS OF AGE

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THREE TEXT FIGURES

INTRODUCTION

The present paper is the first of a series intended to establish vital-capacity standards for Filipinos and is limited to males between the ages of 15 and 30 years. The subjects are mostly university students, and a few laboratory helpers and laborers, who, from the negative findings on medical examination, may be considered as enjoying normal health. The university students were examined in the Department of Physical Education, University of the Philippines, in conjunction with the routine medical examinations conducted annually by the university physician, to whom we wish to express our gratitude for his coöperation and for the privileges extended that made this investigation possible.

APPARATUS AND METHODS

The determinations were made with a carefully calibrated aluminum spirometer, whose light weight made vital-capacity measurements accurate and easy for the subject. The technic consisted in standing directly before the apparatus, taking as deep an inspiration as possible, and then with closed nostrils, delivering all the air in the lungs into the mouthpiece of the spirometer. The procedure was repeated two or three times in each case, and the average of the readings that checked to within 20 cubic centimeters was taken as the vital capacity. This 20 cubic centimeter leeway was allowed because in many nervous subjects closer checks in the same individual were difficult to obtain. The three trials were adopted as a routine because in a large number of cases the first reading tended to be low. The respiratory muscles, like other muscles in the body, require a preliminary "warming up" before reaching optimum efficiency. After the third reading, the effect of fatigue tends to decrease the vital capacity. The heights and weights were taken with the subjects stripped. All records were kept in the metric system, and fractions of a unit were eliminated if less than one-half and taken as one if more than one-half.

PREPARATION OF THE STANDARDS

Several vital-capacity standards have been devised by workers interested in the subject. Myers, in his monograph on vital capacity,(1) finds three fairly satisfactory standards; namely, one based on standing height, one on body weight, and one on body-surface area; the last is considered the best of the three. Other standards are based on formulas developed from measurements of chest circumference, trunk length, etc. It would seem that the best way of gaining an idea of the vital capacity is the measurement of the dimensions of the chest, the box that holds the lungs. Nevertheless, owing to the many factors that intervene, Myers finds that these apparently more-precise standards are no better than the first-named three standards when tested on large numbers of subjects.

We have prepared two standards; namely, one based on standing height, and one based on a combination of standing height and body weight. It is felt that these two standards will be sufficient for most practical purposes. The standing-height standard, though less accurate, may be more handy because meter sticks for height measurements are in general more available than balances for weighing. The standard based on a combination of height and weight should be resorted to for greater accuracy.

1. *Standard based on standing height.*—The vital-capacity determinations on our 2,600 subjects were arranged according to height. If all the 2,600 vital-capacity values were plotted against height it would be difficult to determine with any degree of precision the slope of the line that represents the mean of the observations, because of the large number of points to be considered. Hence, the first step taken in preparing this standard was to determine the average of all cases falling within height ranges of 10. The results are given in Table 1, and presented graphically in fig. 1. The points fall fairly uniformly about a straight line. The three cases with unusual height and extremely low vital capacities were disregarded.

The formula for a straight-line graph as determined by the method of least squares(2) is $y = a + bx$. Applying this to

TABLE 1.—*Vital capacity and standing height.*

Number of cases.	Height range.	Mean height.	Vital capacity.	
			Actual average.	Standard.
	cm.	*cm.*	*cc.*	*cc.*
10	131–140	137	1,740	1,837
56	141–150	147	2,175	2,295
864	151–160	157	2,861	2,754
1,447	161–170	165	3,164	3,121
220	171–180	173	3,556	3,488
[a]1	-------------------	181	3,350	3,854
[a]1	-------------------	188	3,450	4,175
[a]1	-------------------	198	2,600	4,634
2,600	Mean--------	162	3,064	3,007

[a] Not included in the mathematical calculation of the standard.

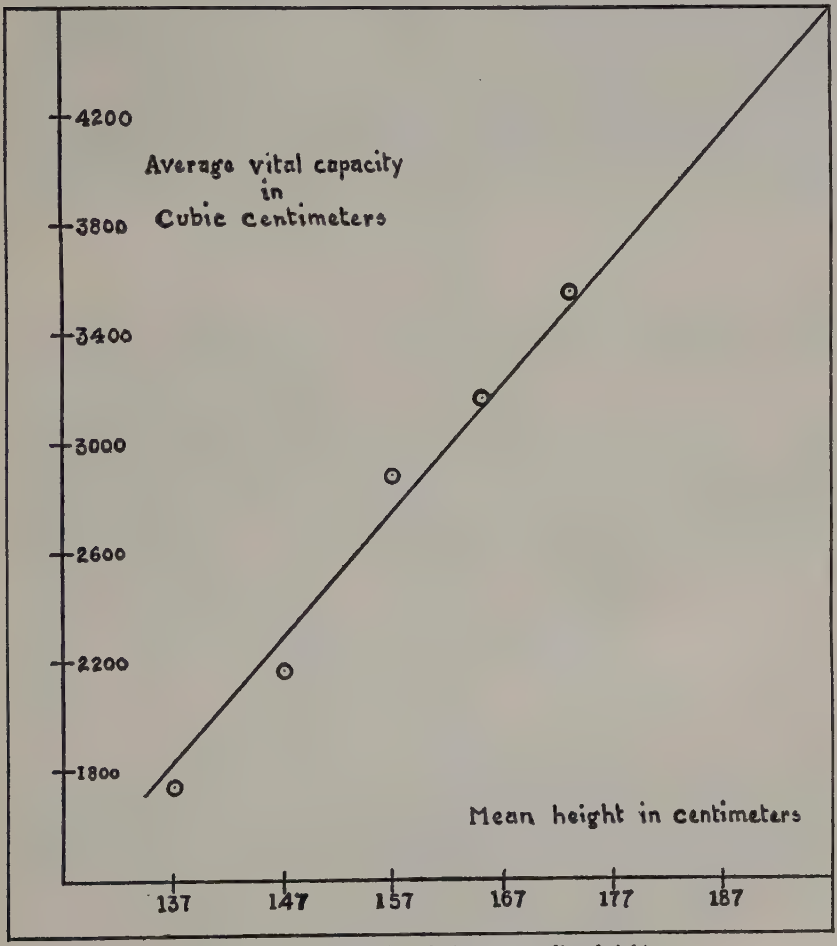

FIG. 1. Vital capacity in relation to standing height.

the data of Table 1 we find $a = -4,444.0$ and $b = 45.85$ where x is the height in centimeters and y is the vital capacity in cubic centimeters.

Whence, V. C. in cc $= 45.85$ ht (cm) $- 4,444.0$. By this formula it is possible to predict roughly the vital capacity when the height in centimeters is known.

2. *Standard based on a combination of height and weight.*—The method of expressing vital capacity in liters per square meter of body surface has been found to be the most satisfactory. We have, however, introduced what we believe to be a simpler and even more-accurate criterion for reckoning vital capacity, based on the product of height in centimeters and weight in kilograms. After all, the Dubois formula(3) for estimating body surface is based on weight and height measurements. Moreover, this formula has been developed from studies on Americans, whose body contours are not the same as those of Filipinos. The height in centimeters was multiplied by the weight in kilograms in each of our cases. For convenience this product will henceforth be designated P. All the P's were arranged in groups of 10 and the average vital capacity for each group was determined. The results are shown in Table 2.

TABLE 2.—*Vital capacity and the product Ht × Wt.*

Number of cases.	Height × weight (P).		Vital capacity.		
	Range.	Mean.	Mean.	In per cent of P.	
				Mean.	Standard.
			cc.		
1	3,100–4,000	3,500	1,528	43.6	43.95
19	4,100–5,000	4,600	1,889	41.0	42.73
56	5,100–6,000	5,600	2,296	41.0	41.39
435	6,100–7,000	6,600	2,720	41.2	40.04
870	7,100–8,000	7,500	3,001	40.0	38.55
718	8,100–9,000	8,500	3,197	37.6	37.20
351	9,100–10,000	9,400	3,418	36.3	35.72
99	10,100–11,000	10,400	3,627	34.8	34.37
34	11,100–12,000	11,500	3,698	32.1	33.15
7	12,100–13,000	12,300	3,657	29.7	31.53
1	----------	13,200	4,300	32.5	30.05
1	----------	14,300	3,500	(a)	----------
1	----------	16,100	4,100	(a)	----------
1	----------	18,500	4,200	(a)	----------
2,600	Mean------	8,000	3,064	----------	----------

[a] Not included in the mathematical calculation of the standard.

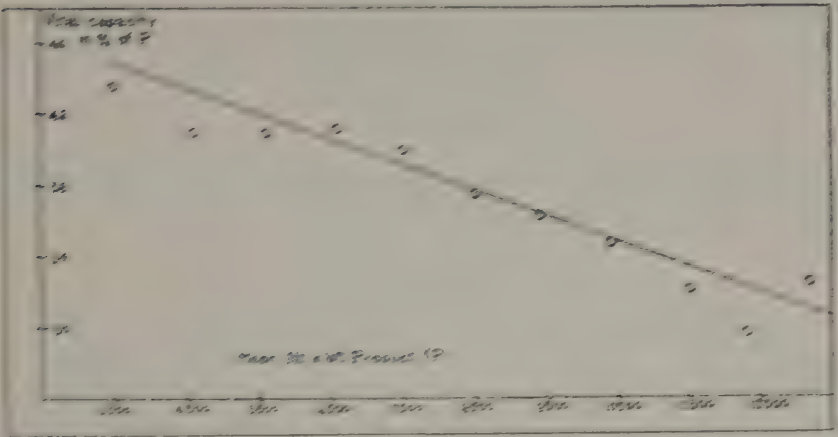

FIG. 2. The relation between the product (P), obtained by multiplying the height in centimeters by the weight in kilograms, and the vital capacity expressed in percentage of this product (per cent of P).

In the fifth column of this table vital capacity is expressed as percentage of P. In fig. 2 vital capacity, as percentage of P, is plotted against P. There is noted a roughly linear tendency to decreasing percentage as P increases. Here again the formula $y = a - bx$ has been applied. If y is taken as the V. C. in per cent of P, and x, as $\frac{P}{1000}$, then $a = 49.0$ and $b = 1.35$. Whence V. C. in per cent of $P = 49 - \frac{1.35\,P}{1000}$. Therefore, V. C. in $cc = \frac{P}{100}\,49 - \frac{1.35\,P}{1000}$.

When the height in centimeters and the weight in kilograms are known, the vital capacity may be readily predicted. For instance, the vital capacity of a man 163 centimeters high and weighing 49 kilograms may be determined as follows: $P = 49 \times 163 = 7{,}987$.

Substituting in the formula

$$\begin{aligned} V.\,C. &= 79.87\,(49 - 1.35 \times 7.987) \\ &= 79.87\,(49 - 10.78245) \\ &= 79.87 \times 38.21755 \\ &= 3{,}052.4357185 \text{ cubic centimeters.} \end{aligned}$$

In practice 10 cubic centimeters, more or less, of vital capacity is of no account. Hence, to make the calculation simpler it is advisable to cut the decimal places to one before starting any multiplication, exception being made of the constant 1.35.

Thus, the above calculation becomes

$$\begin{aligned} \text{V. C.} &= 79.9\ (49 - 1.35 \times 8.0) \\ &= 79.9\ (49 - 10.8) \\ &= 79.9 \times 38.2 \\ &= 3{,}052.18 \end{aligned}$$

For convenience in routine examinations, Table 3 has been prepared to save time and effort.

THE AGE FACTOR

In compiling our data according to the height and weight arrangement we found different ages represented. It is natural to inquire whether the age factor will have to be reckoned with in applying these tables; in other words, whether in individuals of the same Ht × Wt product the vital capacity will vary with age. In Table 4 are given all the cases under $P = 8{,}500 - 8{,}600$ as an illustration. The cases are arranged according to increasing age. The vital capacities exhibit neither an upward nor a downward trend. Most of the other groups under the different Ht × Wt products likewise showed lack of any definite trend. In a few groups vital capacity seemed to increase with increasing age and in about an equal number of groups the vital capacity decreased with increasing age. We are led to conclude that in the age range of 15 to 30 years here represented the age factor is negligible. Bowen and Platt (4) analyzed one hundred eighty-five men and women varying in age from 15 to 85 years, reducing the vital capacities in terms of cubic centimeter per square meter of body surface. They found that up to 30 years of age no change in vital capacity occurred, and that from that age upwards a gradual decline

TABLE 4.—*Age and vital capacity for* $P = 8{,}500 - 8{,}600$

Number of cases.	Age.	Average vital capacity.	Number of cases.	Age.	Average vital capacity.
	Yrs.			Yrs.	
5	15	3,100	5	23	3,340
8	18	3,387	5	24	3,520
13	19	3,169	1	25	3,000
11	20	3,299	2	27	2,800
15	21	3,186	2	29	3,450
10	22	3,229			

170	171	172	173	174	175	176	177	178
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3,361	-------	-------	-------	-------	-------	-------	-------	-------
3,402	3,415	3,428	-------	-------	-------	-------	-------	-------
3,441	3,454	3,467	3,480	-------	-------	-------	-------	-------
3,481	3,495	3,508	3,521	3,535	-------	-------	-------	-------
3,519	3,532	3,546	3,559	3,572	3,585	-------	-------	-------
3,557	3,570	3,584	3,597	3,610	3,623	3,636	3,641	-------
3,593	3,606	3,619	3,632	3,645	3,657	3,670	3,683	3,695
3,630	3,643	3,655	3,668	3,681	3,694	3,707	3,720	3,733
3,665	3,678	3,690	3,703	3,716	3,729	3,742	3,754	3,767
3,699	3,712	3,724	3,737	3,749	3,761	3,773	3,786	3,798
3,733	3,746	3,758	3,770	3,783	3,795	3,807	3,819	3,832
3,766	3,778	3,791	3,803	3,815	3,827	3,840	3,852	3,864
3,798	3,810	3,822	3,835	3,847	3,859	3,871	3,883	3,895
3,829	3,842	3,854	3,866	3,877	3,889	3,901	3,913	3,924
3,861	3,872	3,884	3,896	3,908	3,919	3,931	3,942	3,953
3,890	3,902	3,914	3,926	3,937	3,949	3,960	3,971	3,982
3,919	3,931	3,943	3,954	3,966	3,977	3,988	3,999	4,010
3,948	3,959	3,970	3,982	3,993	4,004	4,015	4,025	4,036
3,975	3,987	3,998	4,009	4,020	4,031	4,042	4,053	4,063
4,002	4,013	4,024	4,035	4,046	4,057	4,068	4,078	4,088
4,028	4,039	4,050	4,061	4,071	4,082	4,092	4,102	4,112

in vital capacity was distinctly manifest. In our present series, therefore, we may disregard the age factor.

VALIDITY OF OUR PREDICTION STANDARD

As has been stated before, the standard based on body surface area is conceded to be the best for vital-capacity work so far devised. Therefore, although we feel that it is not proper to apply the Dubois formula, developed from American subjects, to our series, we felt that it would be interesting to see how our formula would compare with the body-surface standard when the latter is applied to Filipinos. In using the body-surface-area standard it is necessary to determine the average number of liters of vital capacity per square meter of body surface, in other words, the constant. In our series this constant is 2.004, or practically 2. The formula for our series according to the body-surface-area standard is, therefore, V. C. in liters $= 2A$, where A is the body-surface area in square meters.

TABLE 5.—*Frequency distribution of vital-capacity values according to three standards.*

Basis of standard.	Less than 80 per cent of the standard.	80 to 89 per cent of the standard.	90 to 109 per cent of the standard.	110 to 119 per cent of the standard.	Greater than 120 per cent of the standard.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Height	5	13	54	19	9
Ht × Wt	5	14	63	14	4
Body surface	4	12	60	18	6

The percentage distribution of the Filipino cases according to the standards already discussed is given in Table 5 and presented graphically in fig. 3. One sees at a glance that the standard based on our formula is better than that based on body-surface area as applied to the Filipinos.

A comparison was next made between the body-surface formula and ours when applied to American subjects. For this purpose the data of West(5) for men were utilized. Our formula as developed for the American series is $\text{V. C.} = \frac{P}{100}\left(52.7 - \frac{P}{1,000}\right)$. The body-surface formula as applied by West to the same series is $\text{V. C.} = 2.5\ A$. The percentage distribution of the cases in this American series according to the two

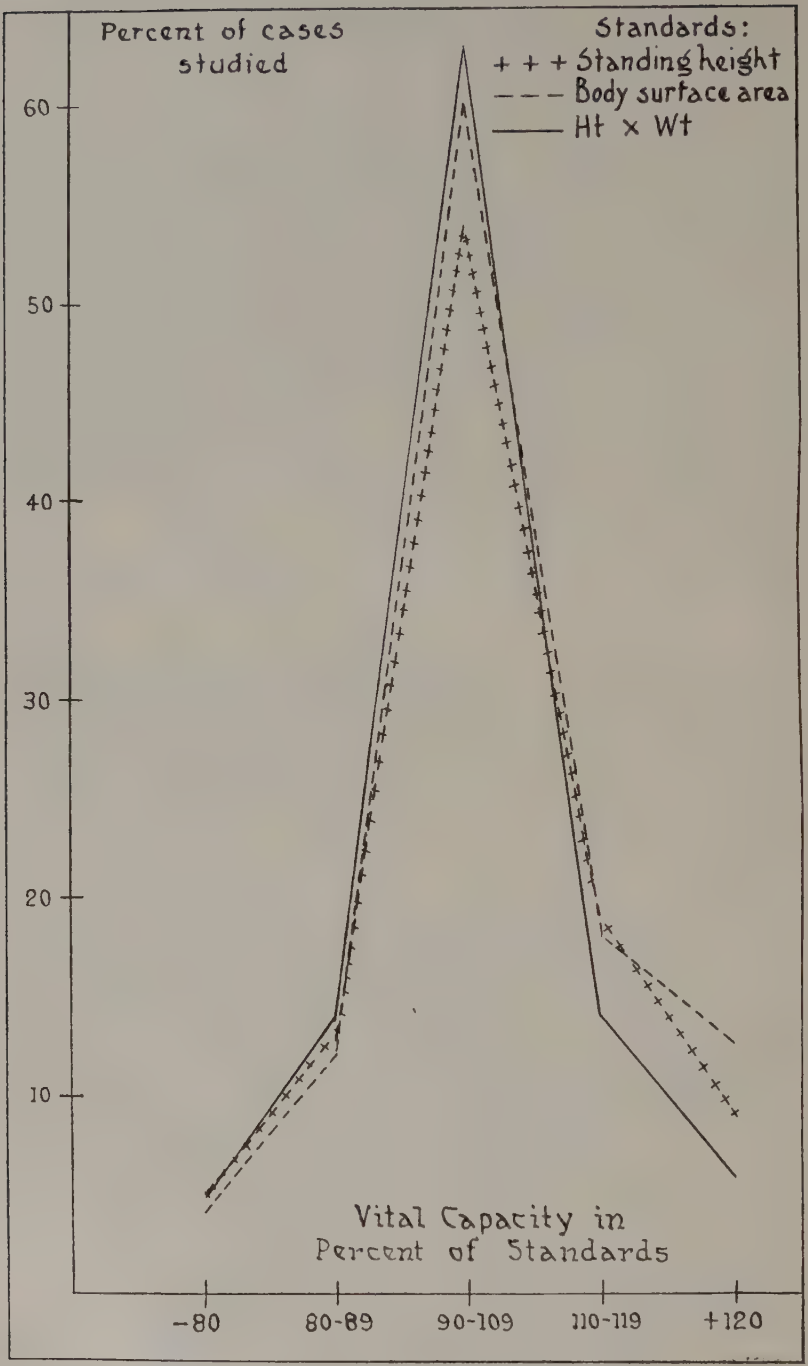

FIG. 3. Percentage distribution of the cases according to three standards.

formulæ is given in Table 6. Even here, our formula seems to give better correlations than the body-surface standard.

TABLE 6.—*Percentage distribution of West's 85 male Americans according to the Ht × Wt (P) and the body-surface standards.*

Basis of standard.	Less than 80 per cent of the standard.	80 to 89 per cent of the standard.	90 to 109 per cent of the standard.	110 to 119 per cent of the standard.	Greater than 120 per cent of the standard.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Body surface	----------	5.9	67.0	22.4	4.7
Ht × Wt (P)	1.2	10.6	74.1	14.1	----------

As may be seen, our formula is developed by a direct statistical study of the height and weight data on a large series of subjects. The body-surface standard is based on two separate statistical studies; one on body-surface area, and the other on vital capacity in relation to body surface. In the latter instance, vital capacity is also correlated with the height and weight but through the intermediation of a statistically determined height-weight formula. It is very probable that the better correlation shown by our formula is not merely accidental. It would be interesting to have it tried out further among American and European subjects.

COMMENTS

The variation in vital capacity from individual to individual is so great even among those of apparently the same general physical condition that it is customary to employ all known standards in determining doubtful cases. It should be borne in mind that most if not all conditions tending to increase vital capacity above normal are favorable for good health, and most of the causes of its reduction are not favorable. From a medical standpoint, therefore, we are interested mostly in persons who fall below the normal average. As has been stated before, all our subjects appeared normal from a routine medical examination. However, most of those whose vital capacities were lower than 80 per cent of the normal were rather below par in physical development. Individuals of this type, constituting 5 per cent of our series, should put us on guard, and they should be carefully examined for possible conditions that may need medical attention. No categorical statement can be made as to what vital capacity is normal and what is abnormal. We have on record a member of our own staff whose vital capacity is about 37 per cent below normal by the different standards, yet he is in a healthier condition than many whose vital capacities are up to standard.

Vital-capacity determinations, though of wide application in medical examinations in America and Europe, are hardly used in this country owing to the lack of standards for the interpretation of the findings. Nañagas(6) and Concepcion(7), independently, studied the vital capacity of Filipinos, but limited themselves to the determination of general averages only. We confirm the findings of the latter to the effect that the mean vital capacity of male Filipinos is about 3,000 cubic centimeters, and is much lower than the American and European average. This diminution may be expected in view of the generally smaller size of Filipinos. But the lowering of vital capacity does not seem to be a decrease merely because of the smaller size. Thus, our men give an average vital capacity of 2 liters per square meter of body surface, equivalent only to the average vital capacity of American women. The American men give 2.5 liters per square meter of body surface. Even thus reduced to as near a common denominator as possible, our people give a proportionately much less vital capacity than Americans and Europeans. It is only pertinent to mention here that among Chinese men(8) the vital capacity expressed in liters per square meter of body surface is also 2, which is of the same order of magnitude as ours. It is not within the scope of this work to discuss all the factors that may affect vital capacity, much less to seek an explanation for the lower figures found for our people.

SUMMARY

1. The vital capacities of 2,600 male university students from 15 to 30 years of age are analyzed, and two prediction standards are prepared; one is based on standing height, and the other is based on the product obtained by multiplying the height in centimeters by the weight in kilograms.

2. The Ht × Wt standard is considered the better of the two. It is also shown that this standard is better than the surface-area standard when applied both to Filipino and American men.

3. The average vital capacity of Filipinos, expressed in liters per square meter of body surface, is 2, the same as that of male Chinese, lower than that of American men (2.5), and equivalent only to the vital capacity of American women.

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ILLUSTRATIONS

TEXT FIGURES

FIG. 1. Chart showing vital capacity in relation to standing height.
2. Chart showing the relation between the product (P) obtained by multiplying the height in centimeters by the weight in kilograms and the vital capacity expressed in percentage of this product (per cent of P).
3. Chart showing percentage distribution of the cases according to three standards.

NEW OR LITTLE-KNOWN TIPULIDÆ FROM EASTERN ASIA (DIPTERA), IX[1]

By CHARLES P. ALEXANDER
Of Amherst, Massachusetts

TWO PLATES

The crane flies discussed herein are chiefly from Szechwan Province, western China, where two important lots of material are included, one from Mount Omei, collected by a Canadian missionary and received through the kind interest of my friend Mr. Herbert S. Parish, the second collected by Reverend David C. Graham and now preserved in the United States National Museum. An exceptionally interesting series of crane flies taken in the Japanese Alps, on and near Mount Norikura, were sent to me by Prof. Jiro Machida, to whom I express my deep thanks for continued coöperation in making known the rich tipulid fauna of the Japanese mountains. One further species from Formosa was included in rich collections taken by my friend, Prof. Syuti Issiki. Except where noted to the contrary, the types of the species here discussed are preserved in my collection.

The following records of species taken in July, 1929, on and near Norikuradake, Shinano, Japan, by Prof. Jiro Machida, are of interest.

Norikuradake, July 26, 1929 (*J. Machida*).

Tipula depressa sp. nov.
Tipula kuzuensis Alex.
Tipula nippoalpina sp. nov.
Tipula politostriata sp. nov.
Tipula sachalinensis Alex.
Tipula uenoi Alex.
Nephrotoma saghaliensis Alex.
Cylindrotoma japonica Alex.
Phalacrocera megacauda sp. nov.
Limonia (*Limonia*) *machidai* (Alex.).
Dicranoptycha cæsia pallidibasis subsp. nov.
Tricyphona pectinata sp. nov.
Tricyphona seticauda sp. nov.
Heterangæus laticincta sp. nov.
Limnophila (*Tricholimnophila*) *saitamæ* Alex.
Limnophila mundella sp. nov.
Molophilus ferox sp. nov.

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[1] Contribution from the Department of Entomology, Massachusetts Agricultural College.

Shirahone Hot Springs, near Norikuradake, July 25, 1929 (*J. Machida*).

Tipula bubo Alex.
Gnophomyia tristis Alex.

Hirayu, Norikuradake, July 27, 1929 (*J. Machida*).

Tipula matsumuriana Alex.
Tipula politostriata sp. nov.
Nephrotoma saghaliensis Alex.
Limonia (*Limonia*) *subnubeculosa* (Alex.).
Heterangæus laticincta sp. nov.
Limnophila (*Ephelia*) *dietziana* Alex.
Erioptera (*Ilisia*) *machidai* sp. nov.

TIPULINÆ

DOLICHOPEZA (NESOPEZA) SUBGENICULATA sp. nov.

Closely allied to *D.* (*N.*) *geniculata* (Alexander) but details of male hypopygium quite distinct; outer dististyle slender, constricted and bent at near midlength; lateral portions of ninth sternite strongly produced caudad.

Male.—Length, about 6.5 millimeters; wing, 8.

Female.—Length, about 8 millimeters; wing, 9.

Antennal flagellum yellow, the outer segments darkened.

Mesonotal præscutum brown with four darker brown stripes that are little evident against the ground color; scutellum and postnotal mediotergite more testaceous. Pleura chiefly dark brown, the dorsopleural region and pteropleurite abruptly pale. Halteres yellow, the knobs blackened. Legs with the fore coxæ brownish black, the remaining coxæ pale; trochanters yellow; femoral tips broadly blackened, the tibial bases narrowly whitened, the tips more broadly blackened; both femora and tibiæ with a more whitish subterminal ring; tarsi white. Wings with the pattern as in *geniculata;* ground color gray, the costal margin with four brown areas, the largest basal, the third along the cord to M, the fourth apical, interrupted by hyaline droplets in outer ends of cells R_3 and R_5; a more whitish border delimiting the brown costal border behind; no conspicuous darkening caudad of the main costal pattern excepting a small cloud at end of Cu_1. Venation: Medial forks short.

Abdomen dark brown, the tergites variegated with gray areas on lateral margins of basal rings; outer sternites more blackened. Male hypopygium with the caudal margin of the ninth tergite (Plate 2, fig. 21) trilobed, the median lobe shortest, obtuse. Outer dististyle (Plate 2, fig. 22) slender, strongly constricted and strongly bent at just beyond midlength. Inner dististyle (Plate 2, fig. 23) broad, near base on outer face with several strong setæ, in addition to the usual more delicate ones. Lateral lobes of ninth sternite strongly developed, stouter than in *oberon.*

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 4,500 feet, July 31, 1929 (ex Parish). Allotopotype, female, August 14, 1929.

Very closely related to *D.* (*N.*) *geniculata* of Japan, differing especially in the structure of the male hypopygium. The production of the lobes of the ninth sternite is much as in *D.* (*N.*) *oberon* (Alexander) of Luzon, but the other features of the hypopygium are distinct.

TIPULA EXUSTA sp. nov.

Belongs to the *cinereifrons* group; thorax and abdomen entirely reddish orange; head gray; antennæ and legs black; R_{1+2} atrophied; male hypopygium with only a single complicated dististyle.

Male.—Length, about 12 millimeters; wing, 11.

Frontal prolongation of head gray, the nasus short and stout; palpi black. Antennæ (male) of moderate length, if bent backward scarcely attaining the wing root; black, the second scapal segment a little paler apically; flagellar segments only slightly enlarged basally. Head dark gray.

Thorax entirely reddish orange, without markings. Halteres pale, the knobs dark brown. Legs with the coxæ and trochanters orange; legs black, the femora narrowly obscure yellow at base. Wings (Plate 1, fig. 1) gray, the stigma brown; obliterative areas very restricted; veins black. Venation: R_{1+2} entirely atrophied; cell 1st M_2 long-pentagonal; petiole of cell M_1 about one-half the cell; cell 2d A relatively wide.

Abdomen, including the hypopygium, reddish orange. Male hypopygium (Plate 2, fig. 24) with the tergite (Plate 2, fig. 25) pale, the caudal margin with a broad, shallow, V-shaped notch, the margins medially narrowly blackened, beneath further produced on either side of the median line into a smooth flattened black plate that is directed caudad and slightly ventrad, the margin obtuse. Basistyle produced caudad into a small obtuse lobule. A single complex flattened dististyle (Plate 2, fig. 26) shaped as in the figure. Notch of the ninth sternite relatively shallow, with a small more or less bifid fleshy lobe jutting caudad, placed close to the suture of the basistyle. Eighth sternite, 8*s*, unarmed.

Habitat.—China (Szechwan).

Holotype, male, Mupin, altitude 3,500 feet, June 30, 1929 (*D. C. Graham*). Type in the collection of the United States National Museum.

Tipula exusta is distinct from regional small species of the *cinereifrons* group in the structure of the male hypopygium.

TIPULA NIPPOALPINA sp. nov.

General coloration gray; nasus lacking; head with a median brown vitta; præscutum with four brown stripes; legs reddish, the tips of the femora and tibiæ narrowly blackened; wings whitish yellow, with a gray and brown clouded pattern; R_{2+3} in direct alignment with the straight R_3; abdomen reddish yellow, segments five to nine black; male hypopygium with the tergite with a U-shaped median notch, and having a smaller quadrate median incision at its base; eighth sternite unarmed.

Male.—Length, about 15 millimeters; wing, 15.5.

Frontal prolongation of head black, sparsely pruinose; nasus lacking; palpi black. Antennæ with the scapal segments and all but the extreme apex of the first flagellar segment reddish yellow; remainder of antennæ black; flagellar segments moderately incised, the longest verticils a trifle shorter than the segments. Head gray, with a pale brown median vitta.

Mesonotal præscutum gray, with four scarcely indicated brown stripes, the intermediate pair obsolete on anterior portions; scutum gray, the lobes very slightly darkened; scutellum and postnotum gray with a nearly continuous median darker vitta. Pleura gray, the dorsopleural region more buffy. Halteres pale, the knobs infuscated. Legs with the coxæ brownish gray; trochanters reddish; femora and tibiæ reddish yellow, the tips narrowly but conspicuously blackened; tarsi passing into black. Wings (Plate 1, fig. 2) with the ground color whitish yellow, the base and costal region clearer yellow; a handsome clouded brown and gray pattern; the ground color includes areas before and beyond the stigma; at beyond midlength of cell R; an oblique area beyond the cord, extending from costa into cell R_5 and a second area extending from the outer end of cell R obliquely across cell 1st M_2 almost to the margin in cell M_4; other pale areas in bases of cubital and anal cells. Venation: Rs long; R_{1+2} subobsolete on outer half; R_2 elongate; R_{2+3} in alignment with the straight R_3.

Abdomen reddish yellow, the basal tergite more pruinose; lateral and caudal portions of segments insensibly darkened;

posterior half of fifth, and all succeeding segments, black. Male hypopygium (Plate 2, fig. 27) with the tergite, sternite, and basistyle all distinct. Ninth tergite (Plate 2, fig. 28) moderately chitinized, not heavily blackened, the caudal margin with a U-shaped notch, at the base of which is a smaller quadrate median incision. Outer dististyle a dusky subcylindrical lobe, the apex obtuse. Inner dististyle (Plate 2, fig. 27, *id*) flattened, compressed, the base with two small lobes, the apex bidentate. Ædeagus, *a*, jutting from genital chamber. Ninth sternite, 9*s*, with a V-shaped incision that extends to the base of the segment, the surface unarmed with lobes or tufts. Eighth sternite unarmed.

Habitat.—Japan (Honshiu).

Holotype, male, Norikuradake, Japanese Alps, Shinano, July 26, 1929 (*J. Machida*).

Tipula nippoalpina is distinct from all similar regional species in the lack of a nasus, details of venation, and structure of the male hypopygium.

TIPULA DEPRESSA sp. nov.

General coloration light brown, the præscutum with almost concolorous stripes that are narrowly margined with darker brown; wings brown and gray, variegated with extensive cream-colored areas; male hypopygium with the ninth tergite tridentate; eighth sternite with a conspicuous median tonguelike lobe.

Male.—Length, about 12 millimeters; wing, 14. Described from a somewhat teneral type specimen.

Frontal prolongation of head brownish testaceous. Antennæ with the first scapal segment dark brown, the tip and second scapal segment more reddish brown; flagellum apparently almost uniformly infuscated, the basal enlargements of the segments little if any darker. Head brown.

Mesonotal præscutum light brown, the four usual stripes almost concolorous, the intermediate stripes margined laterally with a conspicuous darker brown line, the mesal edge of the lateral stripes similarly darkened; posterior sclerites of mesonotum light brown, the scutellum more testaceous. Pleura light brown, the ventral pleurotergite darker. Halteres pale, the knobs broken. Legs with the coxæ light brown, trochanters yellow; remainder of legs brown, the femoral bases more brightened. Wings relatively broad, the ground color brown or gray, variegated by extensive cream-colored areas, including a broad crossband beyond the stigma and cord; other areas before stigma, at base and outer end of cell M, outer ends of radial cells, as a broad seam along vein 1st A in cells Cu and 1st A and in the anal cells; veins brown, paler in the creamy areas. Venation: R_{1+2} entire but with macrotrichia confined to basal two-thirds; R_3 elongate, gently arcuated and thus not in alignment with R_{2+3}; cell 1st M_2 small, pentagonal.

Abdomen chiefly reddish yellow. Male hypopygium with the caudal margin of the ninth tergite (Plate 2, fig. 29) conspicuously trifid, the lobes blackened; lateral lobes flattened, slightly incurved, the tips truncated, the outer angles a little decurved; median lobe narrowed outwardly, the ventral surface with a median keel that juts caudad slightly beyond the level of the lobe. Outer dististyle (Plate 2, fig. 30) with the stem narrowed, the apex expanded into a setiferous foot-shaped structure. Inner dististyle (Plate 2, fig. 31) of unusual form, being deeply bilobed, the anterior or cephalic portion being separated from the basal lobe by a conspicuous rounded notch. Eighth sternite (Plate 2, fig. 32) with a conspicuous median depressed lobe, this bearing a small lateral lobule or shoulder on either side; the median tonguelike lobe beyond the lateral lobules is provided with abundant setæ that are directed mesad, these becoming progressively smaller and more delicate away from the margin. Gonapophyses (Plate 2, fig. 33) bifid, the outer arms appearing as acute blackened hooks.

Habitat.—Japan (Honshiu).

Holotype, male, teneral, Norikuradake, Japanese Alps, Shinano, July 26, 1929 (*J. Machida*).

The structure of the male hypopygium serves to separate this alpine species from similar forms.

TIPULA POLITOSTRIATA sp. nov.

Allied to *T. shomio* Alexander; mesonotal præscutum and scutum gray, variegated with polished black areas; blackened femoral tips narrow, occupying about the distal fourth of the segment, the amount subequal on all legs; wings yellow, especially the base and costal margin, the dark pattern restricted; male hypopygium with the outer dististyle relatively small.

Male.—Length, about 13.5 to 14 millimeters; wing, 15.

Female.—Length, about 17 to 18 millimeters; wing, 15 to 16.

Frontal prolongation of head black, sparsely pruinose, paler laterally; nasus distinct; palpi black. Antennæ (male) of moderate length, if bent backward extending about to the wing root; scape and basal two flagellar segments yellow; succeeding segments almost uniformly darkened or with the bases a little darker; outer verticils much longer than the ventral pair. Antennæ of female more uniformly pale. Head gray.

Pronotum blackish gray. Mesonotal præscutum gray, the three usual stripes polished black, distinctly separated by lines of the ground color; scutum gray, the centers of the lobes polished black; scutellum brownish black; postnotum black, sparsely pruinose. Pleura gray. Halteres pale, the knobs weakly infuscated. Legs with the coxæ black, pruinose; trochanters yellow; femora yellow, the tips broadly blackened, the amount subequal on all legs, occupying approximately the distal fourth of the segment; tibiæ yellow, the tips blackened; tarsi black, the proximal ends of basitarsi restrictedly pale. Wings yellow, the base and costal region clearer yellow; a restricted brown pattern, including the stigma and a seam on the anterior cord; narrower brown seams on posterior cord and along vein Cu; a triangular dusky area in outer end of cell 1st A; wing apex more or less darkened, in cases leaving a poorly indicated area of the ground color distad of the stigma and cord. Venation: R_{1+2} preserved; petiole of cell M_1 subequal to or shorter than second section of M_{1+2}.

Abdominal tergites yellow, with three nearly entire blackish stripes, the lateral stripes wider than the median one, which is more or less interrupted at the caudal margins of the segments; subterminal segments blackened, this including the entire eighth sternite; sternites pale, with a median black stripe, the outer segments blackened; hypopygium chiefly pale. Male hypopygium with the median lobe of the tergite a nearly circular compressed blade; lateral margins of tergite beneath with a short, slender, blackened point. Outer dististyle (Plate 2, fig. 34) relatively small, the head only slightly dilated. Inner dististyle as figured (Plate 2, fig. 35). Gonapophyses (Plate 2, fig. 36) appearing as bispinous plates.

Habitat.—Japan (Honshiu).

Holotype, male, Norikuradake, Japanese Alps, Shinano, July 26, 1929, (*J. Machida*). Allotype, female, Hirayu, July 27, 1929 (*J. Machida*). Paratopotypes, 3 females; paratypes, 4 males and females, with the allotype.

Tipula politostriata is most closely allied to *T. shomio* Alexander and *T. strix* Alexander, differing especially in the more nearly unicolorous wings, without infuscation in the costal region, in the more narrowly darkened femoral tips, and in the details of structure of the male hypopygium.

TIPULA ANGUSTILIGULA sp. nov.

Belongs to the *continuata* group; antennal flagellum entirely black, the segments with a single well-developed verticil; præscutum yellow with three brown stripes, the median stripe prolonged backward onto the median region of the scutum and scutellum; pleura entirely yellow; tips of femora blackened; male hypopygium with the ninth tergite unblackened, the caudal margin with a broad U-shaped emargination; ninth sternite with a narrow median liguliform lobe.

Male.—Length, about 9.5 millimeters; wing, 10.

Frontal prolongation of head relatively short, light yellow on dorsal half, more infuscated laterally; nasus conspicuous; palpi black. Antennæ with the first scapal segment yellow, the second tinged with green; flagellum entirely black; verticils of flagellar segments reduced to a single long basal bristle on outer face. Head yellow, the vertex with a linear more-darkened area.

Pronotum darkened medially, more yellowish laterally. Mesonotal præscutum yellow with three brown stripes, the median stripe narrowed behind and traversing both the scutum and scutellum; scutal lobes and parascutella darkened; postnotal mediotergite brownish yellow, with a clearer yellow median line. Pleura yellow. Halteres dusky, the knobs yellowish. Legs with the coxæ and trochanters yellow; femora obscure yellow basally, passing into dark brown; tibiæ black, the extreme base a little brightened; tarsi black. Wings with the ground color whitish, the base and prearcular region more yellowish; stigma oval, dark brown; conspicuous brown washes and seams along most of the veins, more extensive in the outer radial field and in cells R and M, the latter diversified by a single area of the ground color at about the distal fifth; veins black, the basal and costal regions light brown or yellowish brown. Venation: Rs relatively short, gently arcuated; R_{1+2} entire; cell M_1 very deep, barely sessile to very short-petiolate; second section of M_{3+4} relatively extensive, subequal to or longer than r-m.

Abdomen yellow, becoming more brownish yellow on the outer segments; subterminal segments more uniformly infuscated. Male hypopygium (Plate 2, fig. 37) with the tergite, 9*t*, extensive, not blackened, the caudal margin with a broadly rounded U-shaped notch (Plate 2, fig. 38); on ventral margin at near midwidth of the lobes with a small blackened point. Outer dististyle a long cylindrical rod, the base thicker, the surface with long conspicuous setæ. Inner dististyle with the apex simple. Median notch of the ninth sternite at base with a slender pale lobe (Plate 2, fig. 39), slightly broader at base, gradually narrowed to the blunt tip.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 4,500 feet, August 10, 1929 (ex Parish).

Tipula angustiligula is closest to *T. xanthopleura* Edwards (Tibet to Sikkim); it differs in numerous details, as the entirely blackened antennal flagellum and structure of the male hypopygium, especially the emargination of the tergite and the elongate liguliform lobe of the ninth sternite.

TIPULA LATIFLAVA sp. nov.

Belongs to the *mutila* group; general coloration gray, the præscutum with four broken brown stripes, the interspaces with abundant brown setigerous punctures; knobs of halteres infuscated; legs black, all femora with a very broad yellow subterminal ring; wings whitish subhyaline, with a brown pattern that appears as four interrupted crossbands.

Female.—Length, about 15 millimeters; wing, 13.5.

Frontal prolongation of head gray, more blackened laterally; palpi black. Antennæ with the scape and basal four flagellar segments yellow, the remaining segments more bicolorous, the base being darkened, the apex pale. Head gray, the central region of the vertex with a more or less distinct dusky line.

Pronotum gray, with three brown lines. Mesonotal præscutum gray with four broken brown stripes, the intermediate pair entire on front half, becoming broken behind, separated from one another by a capillary brown median vitta; lateral stripes nearly obsolete, best represented by delicate margins; interspaces with abundant brown setigerous punctures; scutum gray, each lobe with two brown areas; scutellum gray, with a faint brown median vitta; postnotal mediotergite gray with a dark brown median line. Pleura yellowish gray, more or less variegated with darker, especially on the ventral sternopleurite and meron. Halteres yellow, the knobs infuscated. Legs with the coxæ gray; trochanters brownish yellow; femora black, with a very broad yellowish ring before the tips, this ring approximately three times the black tip; tibiæ and tarsi black. Wings (Plate 1, fig. 3) relatively narrow, whitish subhyaline, with a brown pattern that is chiefly distributed as four broken crossbands; cell C dark brown; Sc more yellowish brown; the most extensive of the crossbands is that at origin of Rs, becoming wider and more diffuse in the anal cells; apical band confined to the radial field; narrow brown seams at margin and along veins Cu and 2d A; veins yellow, brown in the clouded areas. Venation: R_{1+2} atrophied; Rs about one-third longer than R_{2+3}; cell 1st M_2 small, irregularly pentagonal, the shortest element being m.

Abdominal tergites obscure brownish yellow, with a conspicuous dorsomedian black stripe that is narrowly interrupted at the caudal margin of each segment; sternites similar, the median stripe broad. Ovipositor with the valves yellowish horn color, the bases of all valves blackened; tergal valves straight and slender.

Habitat.—China (Szechwan).

Holotype, female, Mount Omei, altitude 4,500 feet, August 7, 1929 (ex Parish).

Tipula latiflava is readily told from the two similar regional species, *T. striatipennis* Brunetti and *T. quadrifasciata* Matsumura (*aluco* Alexander), by the unusually broad yellow rings on all femora.

CYLINDROTOMINÆ

CYLINDROTOMA NIGRIPES sp. nov.

General coloration black, the head and thorax more pruinose; dorsopleural region yellow; halteres and legs black; wings with a faint dusky tinge, the small stigma dark brown.

Male?—Length, about 8 millimeters; wings, 9.5.

Mouth parts destroyed. Antennæ short, if bent backward not attaining the wing root; flagellar segments short-cylindrical, the terminal segment longer than the penultimate, pointed at apex; flagellum pale brown, the scapal segments destroyed. Head gray.

Thorax blackened, sparsely gray pruinose, the three præscutal stripes and centers of the scutal lobes more blackened, the interspaces gray pruinose. Pleura black, pruinose, the dorsopleural region conspicuously light yellow; dorsal sternopleurite and ventral pteropleurite a little paler than the remaining pleurites. Halteres blackened. Legs with the coxæ blackish gray, their tips paling to yellow; trochanters obscure yellow; remainder of legs black, only the femoral bases restrictedly obscure yellow. Wings (Plate 1, fig. 4) with a faint dusky tinge, the small oval stigma dark brown; veins dark brown. Venation: Rs long, gently angulated at origin; cell M_1 short-petiolate; m-cu at about two-fifths the lower face of the large cell 1st M_2; cell 2d A relatively narrow.

Abdomen, including the hypopygium, black.

Habitat.—China (Szechwan).

Holotype, sex male?, Mupin, altitude 3,500 feet, June 30, 1929 (*D. C. Graham*). Type in the collection of the United States National Museum.

The antennæ are detached and the tip of the abdomen so crushed that only fragments remain but the sex certainly seems to be male. The black coloration and unusually short antennæ well suffice to distinguish the present species from other known members of the genus.

PHALACROCERA MEGACAUDA sp. nov.

General coloration pale yellow, the mesonotum and pleura variegated with polished black; wings tinged with yellow, the stigma obsolete; basal section of R_{4+5} longer than m-cu, the latter at near two-fifths the length of cell 1st M_2; male hypopygium unusually large, the basistyles apically produced into black earlike plates.

Male.—Length, about 11 millimeters; wing, 10.5.

Rostrum testaceous; palpi pale, the outer segments darkened. Antennæ pale, the outer segments passing into brown. Head brownish black, the anterior vertex silvery.

Pronotum yellow. Mesonotal præscutum yellow with three nearly confluent polished black stripes, the median stripe broadest in front; lateral stripes crossing the suture onto the scutal lobes; remainder of mesonotum yellow, the posterior margin of the postnotal mediotergite with two confluent black areas. Pleura testaceous yellow, the sternopleurite extensively blackened; a smaller black area on the ventral pleurotergite. Halteres chiefly pale. Legs with the coxæ and trochanters yellow; femora obscure yellow, the tips weakly darkened; tibiæ brownish yellow; tarsi passing into dark brown. Wings (Plate 1, fig. 5) tinged with yellow, the stigma obsolete; veins brown. Venation: Sc_1 not reaching costa; free tip of Sc_2 nearly perpendicular, the tip obsolete or nearly so; basal section of R_{4+5} longer than m-cu, the latter gently sinuous, placed at near two-fifths the length of cell 1st M_2.

Abdominal tergites light brown, the outer segments more darkened; hypopygium chiefly pale; sternites obscure yellow, the subterminal segments more infuscated. Male hypopygium unusually large for a member of the genus, the basistyles produced into conspicuous black earlike plates that are conspicuously fringed with yellow setæ.

Habitat.—Japan (Honshiu).

Holotype, male, Norikuradake, Japanese Alps, Shinano, July 26, 1929 (*J. Machida*).

Phalacrocera megacauda is generally similar to *P. mikado* Alexander, differing especially in the unusually large male hypopygium.

LIMONIINÆ

LIMONIINI

LIMONIA (DICRANOMYIA) SUBPULCHRIPENNIS sp. nov.

Differs from *L.* (*D.*) *pulchripennis* in the darkened wing apex and complete white subapical crossband; darkened areas of costal field extending caudad to vein M.

Male.—Length, about 6.5 millimeters; wing, 7.5.

Female.—Length, about 9.5 millimeters; wing, 8.5.

Rostrum black, pruinose; palpi black. Antennæ with the scapal segments black, the flagellum yellow, only the outer segments more infuscated; flagellar segments oval, becoming more elongate outwardly, the verticils short. Head black, yellowish gray pruinose.

Pronotum black medially, more grayish pruinose laterally. Mesonotal præscutum with the ground color brownish gray with three black stripes, the median one broad and complete, the lateral stripes remote from the suture; scutal lobes black; median region of scutum and the scutellum more grayish; postnotum black, sparsely pruinose. Pleura heavily gray pruinose, striped longitudinally with a narrow blackish area on the anepisternum. Halteres yellow, the knobs black. Legs with the coxæ and trochanters black, more or less pruinose; femora yellow, the tips broadly black; tibiæ yellow, the bases and tips narrowly blackened; basitarsi brownish yellow, the outer segments blackened. Wings (Plate 1, fig. 6) whitish, with a heavy brown pattern of the general type of *pulchripennis;* areas of costal margin continued back to vein M and only slightly narrowed posteriorly; wing apex uniformly darkened, not interrupted by a pale streak in cell R_5, as is the case in *pulchripennis;* a complete white subterminal crossband. Venation: Cell 1st M_2 open or tending to be open by the atrophy or thinning out of the distal section of M_3 (figured as present, though faint).

Abdomen black, the hypopygium obscure yellow. Male hypopygium with the rostral spine shorter than in *pulchripennis,* appressed to the margin of style.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 4,500 feet, July 31, 1929 (ex Parish). Allotopotype, female.

Closely allied to *pulchripennis* (Brunetti), differing especially in the wing pattern and details of structure of the male hypopygium.

LIMONIA (EUGLOCHINA) DIGNITOSA sp. nov.

General coloration dark brown; tarsi entirely snowy white; wings with Rs long, angulated at origin; inner end of stigma not passing far basad of fork of Rs; Cu_2 present as a distinct element that is equal in length to Sc or 2d A.

Male.—Length, about 7.5 to 8 millimeters; wing, 8 to 9.

Female.—Length, about 7 to 7.5 millimeters; wing, 7 to 8.

Rostrum and palpi brown. Antennæ black, relatively long for a member of the subgenus; flagellar segments long-oval, with a dusky glabrous apical neck; each flagellar segment with one very long verticil, these unilaterally arranged. Head black.

Thorax dark brown. Halteres elongate, testaceous, the knobs dark brown. Legs with the coxæ and trochanters brownish testaceous; femora and tibiæ black; tarsi entirely snowy white. Wings (Plate 1, fig. 7) with a strong brownish tinge, somewhat clearer basally; stigma oval, darker brown; veins brownish black. Venation: Sc relatively long, the distance between the tip of Sc_1 and origin of Rs not exceeding twice Rs; Sc_2 variable in length; Rs long, arcuated or angulated at origin, remote from the inner end of the stigma; cell 1st M_2 closed; Cu_2 present as a distinct element to about opposite the end of veins Sc_1 and 2d A

Abdomen brownish black, the basal sternites paler.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 6,000 feet, July 24, 1929 (ex Parish). Allotopotype, female. Paratopotypes, 1 male, 1 female.

Limonia (*Euglochina*) *dignitosa* is distinct from all known species of the subgenus. The length of Rs and its relation to the proximal end of the stigma is approached by *L.* (*E.*) *okinawensis* (Alexander), which differs in the darkened proximal ends of the basitarsi, the loss of vein Cu_2, and the shorter Sc.

ANTOCHA (ANTOCHA) MINUTICORNIS sp. nov.

General coloration ocherous, the præscutum with four brown stripes; antennæ small; wings grayish white, the stigma lacking;

male hypopygium with the outer dististyle blackened, glabrous, here the apex narrowed to an obtuse point.

Male.—Length, about 4.5 millimeters; wing, 5.2.

Female.—Length, about 5.5 millimeters; wing, 5.6.

Rostrum brownish yellow; palpi brownish black. Antennæ small in both sexes, if bent backward ending some distance before the wing root; basal segments pale, the outer segments passing into dark brown; pubescence and verticils of segments short. Head brownish gray.

Mesonotal præscutum ocherous, with four brown stripes, the approximated intermediate pair darker and better delimited; scutum and postnotum gray, the scutellum more ocherous. Pleura obscure brownish ocherous, more or less variegated with darker. Halteres dirty white. Legs with the coxæ ocherous, the fore coxæ a little darker; trochanters obscure yellow; remainder of legs brownish yellow, the tarsi passing into brown. Wings (Plate 1, fig. 8) grayish white, the costal region more yellowish; no indication of a stigma; veins pale. Anal angle of wing prominent. Venation: Rs of moderate length only; m-cu before fork of M.

Abdominal tergites brown, the sternites and hypopygium yellow; a conspicuous dark brown subterminal ring in male, occupying segments seven and eight. Male hypopygium (Plate 2, fig. 40) with the caudal margin of tergite, 9*t*, very gently emarginate. Outer dististyle, *od*, blackened, glabrous, near apex gradually narrowed to an obtuse point. Inner dististyle, *id*, a little longer, pale, with conspicuous setæ. Ædeagus slender, subtended by several pale blades and points, as figured.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 4,500 feet, August 2, 1929 (ex Parish). Allotopotype, female, in copula with type.

Antocha minuticornis has a hypopygium that suggests *A. indica* Brunetti (Himalayas, western China, Malay States) and *A. satsuma* Alexander (Japan). The species differs from *indica* in the small antennæ of both sexes and from *satsuma* in the immaculate wings and details of the hypopygium.

ANTOCHA (ANTOCHA) NEBULIPENNIS sp. nov.

Size large (wing, male, 8 millimeters); general coloration yellowish gray; wings whitish subhyaline, handsomely clouded with gray; male hypopygium with the caudal margin of tergite with a rounded lobe on either side of a small rounded notch;

basistyle with the outer apical angle produced into an obtuse lobe; outer dististyle blackened and acute at tip.

Male.—Length, about 7 millimeters; wing, 8.

Rostrum and palpi destroyed. Antennæ relatively short, black; flagellar segments oval, with short verticils. Head yellowish gray.

Mesonotum yellowish gray, crushed in the unique type, the præscutum apparently without stripes. Pleura yellowish gray. Legs with the coxæ brownish gray; legs relatively long, reddish brown, the outer tarsal segments more darkened. Wings (Plate 1, fig. 9) whitish subhyaline, with a clouded gray pattern, including areas in cell R at near midlength of Rs, along cord and outer end of cell 1st M_2; outer end of vein R_3 and as marginal seams along the longitudinal veins beyond the cord; stigma elongate, brownish gray; veins brown, darker in the infuscated areas. Venation: Rs long; inner end of cell R_3 far basad of cell R_5; cell 1st M_2 closed; m-cu shortly before the fork of M.

Abdomen gray. Male hypopygium (Plate 2, fig. 41) with the tergite, *9t*, transverse, the caudal margin with a small rounded lobe on either side of a similarly formed median notch. Basistyle, *b*, with the outer apical angle produced caudad into an obtuse lobe, the dististyles thus subterminal in position. Outer dististyle, *od*, strongly bent before apex, the tip blackened, narrowed into an acute spine. Inner dististyle, *id*, arcuated, the apex more dilated into a weak spatula that is densely set with short curved spinous setæ. Each gonapophysis appearing as a slender yellow rod.

Habitat.—China (Szechwan).

Holotype, male, Mupin, altitude 3,500 feet, June 30, 1929 (*D. C. Graham*). Type in the collection of the United States National Museum.

Antocha nebulipennis is very distinct from regional species in the large size, wing pattern, and structure of the male hypopygium. *Antocha nebulosa* Edwards (Pahang) has a somewhat similarly clouded wing but is a very different fly.

DICRANOPTYCHA CÆSIA PALLIDIBASIS subsp. nov.

Male.—Length, about 7.5 millimeters; wing, 8.5.

Female.—Length, about 8 millimeters; wing, 9.5.

Generally similar to typical *D. cæsia* Alexander, differing as follows:

Scape of antennæ pale. Abdominal tergites dark brown, the basal sternites paler. Male hypopygium with the outer dististyle gently curved, the outer margin with conspicuous appressed serrations, the apical spine smooth. Inner dististyle stout, angularly bent near midlength, the apex subtruncate. Lateral arms of tergite gently curved, the concave margin microscopically serrulate.

Habitat.—Japan (Honshiu).

Holotype, male, Norikuradake, Japanese Alps, Shinano, July 26, 1929 (*J. Machida*). Allotopotype, female.

PEDICIINI

TRICYPHONA PECTINATA sp. nov.

General coloration reddish brown, the præscutum with three ill-defined blackish stripes; wings faintly suffused with brown; cell R_4 petiolate; medial field of wing beyond cord tending to be pectinate by the atrophy of the basal section of M_3.

Male.—Length, about 5.5 to 6 millimeters; wing, 6.5 to 8.

Rostrum and palpi black. Antennæ brown, 15-segmented; basal flagellar segments small and crowded, the outer segments becoming more attenuate. Head gray; anterior vertex wide, with a circular median depression surrounded by an elevated rim.

Pronotum brownish gray. Mesonotum reddish brown, the præscutum with three ill-defined blackish stripes, the lateral stripes barely indicated; scutellum reddish brown. Pleura blackish gray. Halteres pale, the knobs dusky. Legs with the coxæ and trochanters obscure yellow; femora yellow, the tips passing into brown; tibiæ and tarsi brown. Wings (Plate 1, fig. 10) faintly suffused with brown, the stigmal region diffusely darker brown; veins pale brown. Venation: Cell R_4 petiolate, the petiole subequal to r-m; medial field beyond cord tending to be pectinate by the atrophy of the basal section of M_3, in a few cases with cell 1st M_2 closed or with a mere spur of M_3.

Abdomen black, the hypopygium paler. Male hypopygium about as in *T. insulana* Alexander.

Habitat.—Japan (Honshiu).

Holotype, male, Norikuradake, Japanese Alps, Shinano, July 26, 1929 (*J. Machida*). Paratopotypes, 3 males.

Tricyphona pectinata is obviously allied to *T. insulana* Alexander, differing most evidently in the smaller size, brownish body coloration, tinted wings, and the normally pectinate venation of the medial field of the wing.

TRICYPHONA SETIPENNIS sp. nov.

Allied to *T. vetusta* Alexander; general coloration gray, the præscutum with four brown stripes; apical cells of wing, from Sc_1 to 2d M_2, inclusive, with macrotrichia; male hypopygium with the tergite narrowed outwardly, setiferous; mesal lobe of dististyle narrowed outwardly.

Male.—Length, about 9 millimeters; wing, 12.

Rostrum black, a little paler ventrally; palpi black. Antennæ black, the first scapal segment a little paler. Head dark gray, the anterior vertex paler gray.

Pronotum light gray. Mesonotal præscutum gray with four brown stripes, the intermediate pair long, more confluent in front; scutum gray, the lobes more blackened; scutellum and postnotum pale brown, sparsely pruinose. Pleura brown, sparsely pruinose, the ventral sternopleurite darker. Halteres pale, the knobs weakly infuscated. Legs with the coxæ pale, sparsely pruinose; trochanters yellow; femora yellow, passing into brownish black; tibiæ brown, the tips darker; tarsi brownish black. Wings (Plate 1, fig. 11) yellow, with a sparse brown pattern arranged about as in *vetusta.* Sparse macrotrichia in outer ends of cells Sc_1 to 2d M_2, inclusive. Venation: Cell R_3 petiolate, the petiole subperpendicular, in alignment with r-m.

Abdominal tergites light brown, paler laterally; outer segments and hypopygium brownish black. Male hypopygium with the general structure of *T. seticauda* Alexander. Ninth tergite extensive, gradually narrowed outwardly, the surface conspicuously setiferous. Mesal lobe of dististyle narrowed outwardly, the blunt apex with a group of spinous setæ. In *seticauda,* the mesal lobe of the dististyle is a nearly circular flattened disk, its margin evenly rounded.

Habitat.—Japan (Honshiu).

Holotype, male, Norikuradake, Japanese Alps, Shinano, July 26, 1929 (*J. Machida*).

Tricyphona setipennis is most closely related to *T. vetusta* Alexander and *T. seticauda* Alexander, differing most evidently in the macrotrichia in the extreme outer ends of cells Sc_1 to 2d M_2, and the structure of the male hypopygium, especially of the tergite and mesal lobe of the dististyle.

HETERANGÆUS LATICINCTA sp. nov.

Size large (wing over 10 millimeters); antennal flagellum yellow; thoracic pleura with the anepisternum and sternopleurite darkened; legs yellow, the tips of the femora and tibiæ darkened;

wings whitish with a heavy, chiefly cross-banded pattern, including three crossbands; cell C uniformly darkened; cell 2d A wide, the margin crenulate; supernumerary crossveins as in the genus, that in cell R_3 placed far distad, in cell R_4 about in alignment with R_2, in cell M_1 at two-thirds the length of cell.

Male.—Length, about 9.5 millimeters; wing, 10.5.

Female.—Length, about 10 millimeters; wing, 11.5.

Rostrum and palpi black. Antennæ with the basal segment black, the second segment and flagellum yellow. Head gray; vertical tubercle conspicuous.

Pronotum brown, paler laterally. Mesonotal præscutum grayish brown, the humeral region paler; scutellum and postnotum obscure yellow. Pleura yellow, the sternopleurite and anepisternum darker. Halteres of moderate length, yellow, the knobs only weakly darkened. Legs with the coxæ and trochanters pale yellow; femora yellow, the tips broadly and conspicuously dark brown; tibiæ yellowish white, the tips conspicuously blackened, the amount a little less than the femoral tips; tarsi yellow, the tips of segments one to three and all of the remaining segments black. Wings (Plate 1, fig. 12) whitish, with an unusually heavy, chiefly cross-banded pattern, pale yellowish brown, margined with darker brown; cells C and Sc uniformly dark brown, cell Sc_1 variegated with the ground color; the chief fasciæ are those at origin of Rs, including cells R and M; the cord; and a more irregular fascia at the level of the outer end of cell 1st M_2, sending a ray to the apex in cell R_5 and connected with the central fascia in cells R_5 and Cu; fasciæ nearly parallel-sided and unusually wide; a more or less confluent series of spots in cell Cu; a large confluent area in cells 1st A and 2d A, inclosing a circular pale area near the outer end of cell 2d A; veins yellow, darker in the infuscated areas. Wings relatively broad, especially the anal cells; cell 2d A with the margin strongly crenulate to bilobed before the vein. Venation: Rs square and spurred at origin; R_{2+3+4} short, less than the perpendicular basal section of R_{2+3}; supernumerary crossvein in cell R_3 lying far distad, that of cell R_4 nearly in alignment to just beyond the level of R_2, that of cell M_1 lying beyond two-thirds the length of the cell and in alignment with the crossvein in cell R_3; cell 2d A much wider than in *H. japonicus*.

Abdominal tergites brownish black, a little paler laterally; sternites brighter; male hypopygium dark. Male hypopygium almost as in the other species of the genus, the arm of the dististyle unispinous, the ædeagus capitate.

Habitat.—Japan (Honshiu).

Holotype, male, Hirayu, Japanese Alps, Shinano, July 27, 1929 (*J. Machida*). Allotype, female, Norikuradake, Japanese Alps, July 26, 1929 (*J. Machida*).

Heterangæus laticincta is evidently most closely allied to *H. japonicus* (Alexander) from which it differs in the large size, darkened areas on pleura, and details of venation and wing pattern. The allotype female has a weak adventitious crossvein in cell R_1 immediately distad of the angulation of R_{2+3} and another in cell R_5 just beyond the fork of M_{1+2}.

HEXATOMINI

LIMNOPHILA MUNDELLA sp. nov.

General coloration black, the mesonotum subnitidous; pleura heavily pruinose; halteres yellow; wings brownish yellow, the base and costal region brighter yellow; cell M_1 about twice its petiole; m-cu beyond midlength of cell 1st M_2.

Female.—Length, about 7.5 millimeters; wing, 6.5.

Rostrum and palpi black. Antennæ with the scapal segments black, the flagellum brown; flagellar segments oval, gradually decreasing in size outwardly. Head black, sparsely pruinose, especially in front.

Pronotum black, pruinose. Mesonotum black, subnitidous, the surface only vaguely pruinose. Pleura heavily pruinose. Halteres yellow. Legs with the coxæ and trochanters gray pruinose; remainder of legs broken. Wings (Plate 1, fig. 13) with a strong brownish yellow tinge, the costal and prearcular regions brighter yellow; stigma oval, pale brown, a little deeper than the ground color; veins brown, brighter in the flavous areas. Venation: Sc_1 ending shortly before fork of Rs, Sc_2 near its tip; R_2 a trifle longer than R_{1+2}; cell M_1 about twice its petiole; m-cu beyond midlength of cell 1st M_2

Abdomen with the tergites black, sparsely pruinose; sternites brownish gray. Ovipositor with the valves elongate, blackened.

Habitat.—Japan (Honshiu).

Holotype, female, Norikuradake, Japanese Alps, Shinano, July 26, 1929 (*J. Machida*).

Limnophila mundella is generally similar to the Nearctic *L. munda* Osten Sacken. In the absence of the male sex I hesitate to refer the species to the subgenus *Prionolabis* where it will probably be found to belong. It is readily told from all other Japanese species of *Prionolabis* with cell M_1 present by the small size and unpatterned wings.

LIMNOPHILA NESONEMORALIS sp. nov.

Belongs to the *nemoralis* group; wings gray, in male not evidently widest opposite vein 1st A; male hypopygium with the gonapophyses appearing as spatulate blades.

Male.—Length, about 5 millimeters; wing, 6 to 6.5.

Female.—Length, about 5.5 to 6 millimeters; wing, 5.5 to 6.5.

Rostrum dark brownish gray; palpi black. Antennæ with the basal segment black, the flagellum brown. Head gray, the orbits broadly more whitish gray.

Mesonotum brownish gray, the præscutum with an intermediate pair of darker brown stripes, the lateral stripes scarcely evident. Pleura gray. Halteres relatively elongate, yellow, the knobs very slightly darkened. Legs with the coxæ and trochanters yellow; femora yellow, the tips slightly darkened; remainder of legs yellow, the tarsi deepening to brown; segments of legs rather conspicuously hairy. Wings (Plate 1, fig. 14) of male not noticeably widest opposite vein 1st A, as is the case in *L. subnemoralis;* brownish gray, the stigma a little darker; veins pale brown. Venation: Sc_1 ending about opposite the fork of R_{2+3+4}, Sc_2 a variable distance from its tip; R_2 faint; cell M_1 small, as in the group.

Abdomen brown, the subterminal segments of male not suddenly blackened, as is the case in *subnemoralis.* Male hypopygium (Plate 2, fig. 42) with the outer dististyle, *od*, conspicuously bifid at apex. Gonapophyses, *g*, appearing as flattened spatulate blades, the tips obtusely rounded.

Habitat.—Formosa.

Holotype, male, Shinten, December 3, 1928 (*S. Issiki*). Allotopotype, female. Paratopotypes, 5 males and females.

Limnophila nesonemoralis is very similar to *L. inaequalis* Alexander (Formosa) but has the gonapophyses very different in structure.

ERIOCERA CÆSAREA sp. nov.

Head black; thorax entirely dark brown, opaque; halteres dark brown; wings suffused with brown, deepest along the outer costal margin; cell M_1 deep; cell 1st M_2 small and nearly square; abdominal segments one and five to nine inclusive black, segments two to four orange-yellow.

Male.—Length, about 25 millimeters; wing, 23.5; antenna, about 6.

Rostrum and palpi black. Antennæ 7-segmented, relatively short, as shown by the measurements; flagellar segments decreasing in length and diameter outwardly, densely set with semierect setæ; scapal segments black; flagellum yellowish brown, the outer segments darker. Head dull black; vertical tubercle entire.

Thorax entirely dull chocolate brown to dark brown, the præscutum with four glabrous stripes that are slightly brighter in color. Halteres dark brown. Legs with the coxæ and trochanters dark brown; remainder of legs brownish black (only a single, posterior, leg remains, tarsi broken). Wings (Plate 1, fig. 15) with a strong brown suffusion, the costal region darker brown, especially on the distal half; extensive paler discal streaks before cord; anal cells pale, especially basally; veins brownish yellow. Venation: Sc_1 angulated and weakly spurred at extreme tip; Rs very long, about one-third longer than R; R_{2+3+4} subequal to basal section of R_5; R_{2+3} about two-fifths R_{1+2}; cell M_1 deep, nearly three times its petiole; cell 1st M_2 small and nearly square; m-cu about one-third longer than the distal section of Cu_1, some distance beyond the fork of M.

Abdomen with the basal segment brownish black, its caudal margin narrowly yellow; segments two to four bright orange-yellow; segment five black, the disk suffused with orange; remainder of abdomen, including the hypopygium, velvety black.

Habitat.—China (Szechwan).

Holotype, male, Kwanshien, altitude 1,800 to 3,500 feet, 1924 (*D. C. Graham*). Type in the collection of the United States National Museum.

By Edwards's key [2] the present species runs to couplet 41, agreeing most closely with *E. nigripennis* de Meijere, a quite distinct species. The fly appears to be most nearly allied to the later-described *E. prælata* Alexander, differing in the pattern of the body, wings, and legs.

ERIOPTERINI

NEOLIMNOPHILA PICTURATA sp. nov.

General coloration black, pruinose; halteres yellow; legs black; wings pale cream color, with a heavy dark brown pattern, including broad seams along cord and some of the longitudinal veins, and large rounded clouds at origin of Rs, fork of R_{3+4} and fork of M_{1+2}; r-m connecting with Rs before fork; R_2 nearly twice its own length before the fork of R_{3+4}.

Male.—Length, about 6.5 millimeters; wing, 8.5.

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[2] Ann. & Mag. Nat. Hist. IX 8 (1921) 70–78.

Rostrum and palpi black. Antennæ black throughout; fusion segment involving four segments and a partial fifth; outer flagellar segments elongate-oval, the verticils shorter than the segments. Head dark gray.

Thoracic dorsum black, with a very sparse gray pollen. Pleura more heavily pruinose. Halteres yellow. Legs with the coxæ pruinose; remainder of legs black. Wings (Plate 1, fig. 16) pale cream color, with a heavy dark brown pattern, arranged as follows: Cell C; cell Sc except base and apex; stigma; broad seams along cord and outer end of cell 1st M_2; continuous brown seams along veins R_5, outer sections of veins M_3, M_4, Cu, and 2d A; large rounded clouds at origin of Rs, fork of R_{3+4}, crossing the adjoining cells to vein R_5 and fork of M_{1+2}; the following veins are not seamed with dark, except as noted above: Rs, R_3, R_4, M and its branches, with the exception of the outer sections of M_3 and M_4, and 1st A; prearcular region more yellowish; veins pale yellow, darker in the infuscated areas. Venation: Sc_1 ending about opposite r-m, Sc_2 at its tip; Rs strongly arcuated at origin; r-m connecting with Rs before the fork of latter; R_2 only about one-half R_{3+4}; cell R_3 relatively small, vein R_3 being a little more than one-half R_4; cell 1st M_2 elongate; m-cu about two-thirds its own length beyond the fork of M.

Abdomen, including the hypopygium, black. Male hypopygium with the basistyles broken beyond base.

Habitat.—China (Szechwan).

Holotype, male, Mupin, altitude 3,500 feet, June 30, 1929 (*D. C. Graham*). Type in the collection of the United States National Museum.

The only allied species is *N. fuscinervis* Edwards (Yunnan), which differs in the wing pattern and venation. In addition to the seamed veins of *fuscinervis*, the present species has large rounded clouds at origin of Rs, and at forks of R_{3+4} and M_{1+2}; r-m connects with Rs before the fork and R_2 is far before the fork of R_{3+4}.

TEUCHOLABIS (TEUCHOLABIS) SCITAMENTA sp. nov.

General coloration yellow and blue-black; three nearly confluent black stripes on præscutum, the lateral pair reaching the margins of the sclerite; wings yellowish with a restricted dark pattern; male hypopygium very simple, the styli terminal in position, the outer dististyle a pale ribbonlike blade.

Male.—Length, about 6 millimeters; wing, 5.8 to 6.

Female.—Length, about 6.5 millimeters; wing, 6 to 6.3.

Rostrum brown, a little longer than the remainder of the head; palpi black. Antennæ with the scape yellowish brown; flagellum light brown, the outer segments darker; flagellar segments short-oval, with a dense white pubescence. Head dull black, heavily pruinose, especially in front.

Pronotum orange-yellow. Mesonotal præscutum yellow with three extensive blue-black stripes that are confluent behind or nearly so; lateral stripes reaching the lateral margin of præscutum, leaving a narrow yellow area behind at the suture; humeral region more orange-yellow; median region of scutum and the scutellum testaceous-yellow; scutal lobes blackened; postnotum black. Pleura yellow, the anepisternum, dorsal pteropleurite and the pleurotergite blue-black, the sternopleurite more reddish brown, the two areas separated by a narrow yellow longitudinal stripe that is densely white pubescent. Halteres black, the outer half of knob orange-yellow. Legs with the coxæ and trochanters orange-yellow; fore femora with more than the distal half black, the base yellow; middle femora yellow; posterior femora yellow, the tips narrowly brownish black; fore tibiæ and tarsi black; middle and hind tibiæ yellow, narrowly blackened at bases; tarsi obscure yellow, the apices of segments one and two and all of outer segments blackened. Wings (Plate 1, fig. 17) broad, yellowish, the costal region brighter yellow; stigma brown; a narrow brown seam on anterior cord; a very broad and diffuse slightly darker clouding occupies the central part of the region before the cord; veins yellow, darker in the infuscated areas. Venation: Sc_1 ending at near midlength of Rs, Sc_2 far from its tip; Rs nearly straight on basal half; R_{2+3+4} subequal to or shorter than R_2; vein 2d A curved strongly into the anal margin of wing.

Abdomen black, the bases of the second and succeeding segments in male yellow, the black of the outer tergites sending cephalad a median spur that becomes larger on outer segments so as finally to isolate the yellow to lateral areas on the subterminal segments; hypopygium small, dark brown. Abdominal tergites of female uniformly blackened, only the genital segment orange, the sternites with more yellow. Male hypopygium (Plate 2, fig. 43) unusually small and simple. Basistyle, *b*, short and broad. Outer dististyle, *od*, a pale ribbonlike blade. Inner dististyle, *id*, a flattened blade that terminates in a blackened beak, the outer angle with three long setæ. Phallosome in slide mounts large.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 4,500 feet, August 12, 1929 (ex Parish). Allotopotype, female, August 18, 1929. Paratopotype, female, August 22, 1929.

Teucholabis scitamenta is distinct from all regional species in the features above listed. The structure of the male hypopygium is unusually simple, more so than in any regional species known to me.

ERIOPTERA (ERIOPTERA) BREVIRAMA sp. nov.

General coloration reddish brown; head black, the orbits narrowly marked with silver; pleura iridescent blue; knobs of halteres darkened; wings brownish yellow; male hypopygium with the tergite emarginate; inner dististyle with two divergent arms.

Male.—Length, about 4.6 to 5 millimeters; wing, 4.5 to 5.

Female.—Length, about 5.5 to 6 millimeters; wing, 5.5 to 5.8.

Rostrum and basal segments of palpi yellow, the outer segments darker. Antennæ brownish black, the basal segments a very little paler. Head black, the orbits with a narrow silvery line.

Mesonotum reddish brown, the median region darker, the humeri brighter; scutellum dark brown, narrowly bordered by pale; postnotum dark gray. Pleura almost entirely covered by an iridescent blue pruinosity. Halteres pale, the knobs infuscated. Legs relatively long and stout; coxæ and trochanters yellow; remainder of legs yellow, the terminal tarsal segments infuscated. Wings (Plate 1, fig. 18) with a strong brownish yellow tinge, the costal region clearer yellow; veins light brown, more yellowish in the costal region. Venation: Distal section of Cu_1 and 2d A strongly sinuous on distal portion.

Abdominal segments chiefly dark brown, the caudal margins somewhat brightened; hypopygium obscure yellow. Male hypopygium (Plate 2, fig. 44) with the tergite shallowly emarginate, the median region not convexly produced as in *E.* (*E.*) *ornatifrons.* Dististyles almost as in *ornatifrons,* entirely glabrous, the branches of the inner dististyle, *id,* shorter and more nearly equal, the outer arm not subtended on its basal third by a pale flange but with a conspicuous flattened structure near the fork of the style.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 4,500 feet, July 31, 1929 (ex Parish). Allotopotype, female, August 11, 1929. Paratopotypes, 4 males and females, August 10 to 20, 1929.

Most closely allied to *E.* (*E.*) *ornatifrons* Edwards (Ceylon), differing most evidently in the structure of the male hypopygium.

ERIOPTERA (ILISIA) MACHIDAI sp. nov.

General coloration brown; antennal flagellum brownish yellow; wings yellowish gray, with a very sparse pale brown pattern, including vague clouds at Sc_2, R_2, tip of R_{1+2} and along cord; Sc_1 longer than Rs; cell 1st M_2 moderately elongate; anal veins gradually diverging.

Female.—Length, about 4.5 millimeters; wing, 4.6.

Rostrum and palpi brown. Antennæ with the basal segments dark brown, the flagellum paling to yellow or brownish yellow. Head grayish brown.

Pronotum brown, the anterior lateral pretergites light yellow. Mesonotum brown, the præscutum a little paler laterally. Pleura light brown, the dorsopleural region pale yellow. Halteres pale yellow, the knobs with golden-yellow setæ. Legs with the coxæ and trochanters yellow; femora brownish yellow, the tips a little darkened; tibiæ and tarsi obscure yellow, the latter passing into brown. Wings (Plate 1, fig. 19) yellowish gray, the base and costal region a trifle more yellowish; a very sparse and vague pale brown pattern, including clouds at Sc_2, R_2, and tip of Sc_1, and tip of R_{1+2}, the latter two areas delimiting the ends of a yellowish stigmal area; cord vaguely seamed with dusky; veins very pale brown, the macrotrichia darker. Venation: Sc_1 elongate, exceeding Rs; Sc_2 about opposite one-sixth the length of Rs; cell 1st M_2 moderately elongate; m-cu before the fork of M; vein 2d A nearly straight, the anal veins gradually diverging.

Abdomen dark brown. Ovipositor with the valves very long and slender, pale yellow.

Habitat.—Japan (Honshiu).

Holotype, female, Hirayu, Japanese Alps, Shinano, July 27, 1929 (*J. Machida*).

I take great pleasure in naming this very distinct species in honor of my friend Dr. Jiro Machida, to whom I am indebted for many Japanese Tipulidæ. *Erioptera machidai* is very different from the remaining species of the subgenus *Ilisia* described from eastern Asia. It is more closely allied to *E. maculata* and allies than to the species centering about *E. areolata* Siebke.

ORMOSIA GRAHAMI sp. nov.

Size very large (wing, female, 10 millimeters); general coloration gray, the præscutum with three confluent black stripes; halteres light yellow; legs black; wings white, with a heavy dark brown pattern that appears chiefly as seams along the veins; veins R_3 and R_4 deflected strongly cephalad at outer ends; a weak spur at near two-thirds the length of vein R_4, directed into cell R_4; cell 1st M_2 closed; vein 2d A very strongly sinuous.

Female.—Length, about 8.5 millimeters; wing, 10.

Rostrum and palpi black. Antennæ black throughout; flagellar segments oval, with relatively short verticils, these becoming smaller and less conspicuous on the outer segments. Head blackish, the orbits gray.

Mesonotal præscutum with the disk chiefly covered by three confluent black stripes, the ground color restricted to the humeral and lateral portions; pseudosutural foveæ black; scutum gray, the lobes chiefly blackened; posterior sclerites of mesonotum black, sparsely pruinose. Pleura black, pruinose. Halteres entirely light yellow. Legs with the coxæ black, pruinose; remainder of legs black. Wings (Plate 1, fig. 20) white, with a heavy dark brown pattern; prearcular region and an oval area in cells C and Sc before the stigma light yellow; the brown areas include most of cells C and Sc and very conspicuous seams along most of the longitudinal veins, in cell M_4 so broad as to restrict the ground color to a long-oval area; a cross-fascia in cell 1st A at about two-thirds the length; veins brown, more flavous in the yellow areas. Macrotrichia of cells corresponding in color to the areas occupied, being white, brown, and golden. Venation: R_2 just before fork of R_3 and R_4, the two latter veins deflected strongly cephalad at their outer ends; vein R_3 strongly looped on distal half; R_4 with a spur at two-thirds the length, directed into cell R_4; cell 1st M_2 closed; vein 2d A very strongly sinuous.

Abdomen grayish black, with sparse yellow setæ. Ovipositor with the valves horn yellow, the tergal valves blackened basally.

Habitat.—China (Szechwan).

Holotype, female, Mupin, altitude 3,500 feet, June 30, 1929 (*D. C. Graham*). Type in the collection of the United States National Museum.

Ormosia grahami is named in honor of the Reverend David C. Graham, who has added materially to our knowledge of the fauna and flora of Szechwan and eastern Tibet. It is a strikingly distinct fly that requires no comparison with any known species, being the largest and most conspicuously patterned form so far discovered.

MOLOPHILUS FEROX sp. nov.

Belongs to the *gracilis* group; general coloration light brown; head gray; male hypopygium with unusually complicated armature, including a spinous rod arising from the outer portion of the basistyle, together with three dististyles, one of which is bifid.

Male.—Length, about 4.5 millimeters; wing, 5.

Rostrum and palpi black. Antennæ broken. Head gray.

Pronotum blackish. Mesonotum light brown, the pleura somewhat darker, the ventral sternopleurite and meron more testaceous brown. Halteres chiefly pale, the base of the stem restrictedly yellow, the stem dusky, the large knobs yellowish brown. Legs with the coxæ and trochanters yellowish testaceous; remainder of legs pale brown, the tarsi passing into brownish black. Wings grayish, the veins darker; macrotrichia long, dark brown. Venation: R_2 lying distad of r-m; m-cu a little less than one-half the petiole of cell M_3; vein 2d A of moderate length.

Abdomen brown, the sternites somewhat paler, the conspicuous genitalic styli black. Male hypopygium (Plate 1, fig. 45) with the outer lobe of the basistyle, *b*, produced into a long sinuous rod that is subequal or longer than the dististyle, on outer margin at near one-third the length with an acute yellow spine; distal two-thirds of the rod narrower, the surface more roughened; ventral surface of basistyle with a slender fingerlike lobe. Three dististyles, *d*, the longest a curved sinuous smooth rod, the distal portion bent at about a right angle into a long straight spine. Second dististyle conspicuously bifid. Third dististyle more basal in position, inserted in the notch of the basistyle, a simple blackened rod, the basal half a little thicker, the gently curved distal half narrower. Ædeagus long, narrow, yellow.

Habitat.—Japan (Honshiu).

Holotype, male, Norikuradake, Japanese Alps, Shinano, July 26, 1929 (*J. Machida*).

Molophilus ferox is very distinct from all other regional species of the genus in the structure of the male hypopygium.

ILLUSTRATIONS

[Legend: *a*, ædeagus; *b*, basistyle; *d*, dististyle; *g*, gonapophysis; *id*, inner dististyle; *od*, outer dististyle; *s*, sternite; *t*, tergite.]

PLATE 1

FIG. 1. *Tipula exusta* sp. nov., venation.
2. *Tipula nippoalpina* sp. nov., venation.
3. *Tipula latiflava* sp. nov., venation.
4. *Cylindrotoma nigripes* sp. nov., venation.
5. *Phalacrocera megacauda* sp. nov., venation.
6. *Limonia (Dicranomyia) subpulchripennis* sp. nov., venation.
7. *Limonia (Euglochina) dignitosa* sp. nov., venation.
8. *Antocha (Antocha) minuticornis* sp. nov., venation.
9. *Antocha (Antocha) nebulipennis* sp. nov., venation.
10. *Tricyphona pectinata* sp. nov., venation.
11. *Tricyphona setipennis* sp. nov., venation.
12. *Heterangæus laticincta* sp. nov., venation.
13. *Limnophila mundella* sp. nov., venation.
14. *Limnophila nesonemoralis* sp. nov., venation.
15. *Eriocera cæsarea* sp. nov., venation.
16. *Neolimnophila picturata* sp. nov., venation.
17. *Teucholabis (Teucholabis) scitamenta* sp. nov., venation.
18. *Erioptera (Erioptera) brevirama* sp. nov., venation.
19. *Erioptera (Ilisia) machidai* sp. nov., venation.
20. *Ormosia grahami* sp. nov., venation.

PLATE 2

FIG. 21. *Dolichopeza (Nesopeza) subgeniculata* sp. nov., male hypopygium, ninth tergite.
22. *Dolichopeza (Nesopeza) subgeniculata* sp. nov., male hypopygium, outer dististyle.
23. *Dolichopeza (Nesopeza) subgeniculata* sp. nov., male hypopygium, inner dististyle.
24. *Tipula exusta* sp. nov., male hypopygium, lateral aspect.
25. *Tipula exusta* sp. nov., male hypopygium, ninth tergite.
26. *Tipula exusta* sp. nov., male hypopygium, dististyle.
27. *Tipula nippoalpina* sp. nov., male hypopygium, lateral aspect.
28. *Tipula nippoalpina* sp. nov., male hypopygium, ninth tergite.
29. *Tipula depressa* sp. nov., male hypopygium, ninth tergite.
30. *Tipula depressa* sp. nov., male hypopygium, outer dististyle.
31. *Tipula depressa* sp. nov., male hypopygium, inner dististyle.
32. *Tipula depressa* sp. nov., male hypopygium, lobe of eighth sternite.
33. *Tipula depressa* sp. nov., male hypopygium, gonapophysis.

FIG. 34. *Tipula politostriata* sp. nov., male hypopygium, outer dististyle.
35. *Tipula politostriata* sp. nov., male hypopygium, inner dististyle.
36. *Tipula politostriata* sp. nov., male hypopygium, gonapophysis.
37. *Tipula angustiligula* sp. nov., male hypopygium, lateral aspect.
38. *Tipula angustiligula* sp. nov., male hypopygium, ninth tergite.
39. *Tipula angustiligula* sp. nov., male hypopygium, lobe of ninth sternite.
40. *Antocha (Antocha) minuticornis* sp. nov., male hypopygium.
41. *Antocha (Antocha) nebulipennis* sp. nov., male hypopygium.
42. *Limnophila nesonemoralis* sp. nov., male hypopygium.
43. *Teucholabis (Teucholabis) scitamenta* sp. nov., male hypopygium.
44. *Erioptera (Erioptera) brevirama* sp. nov., male hypopygium.
45. *Molophilus ferox* sp. nov., male hypopygium.

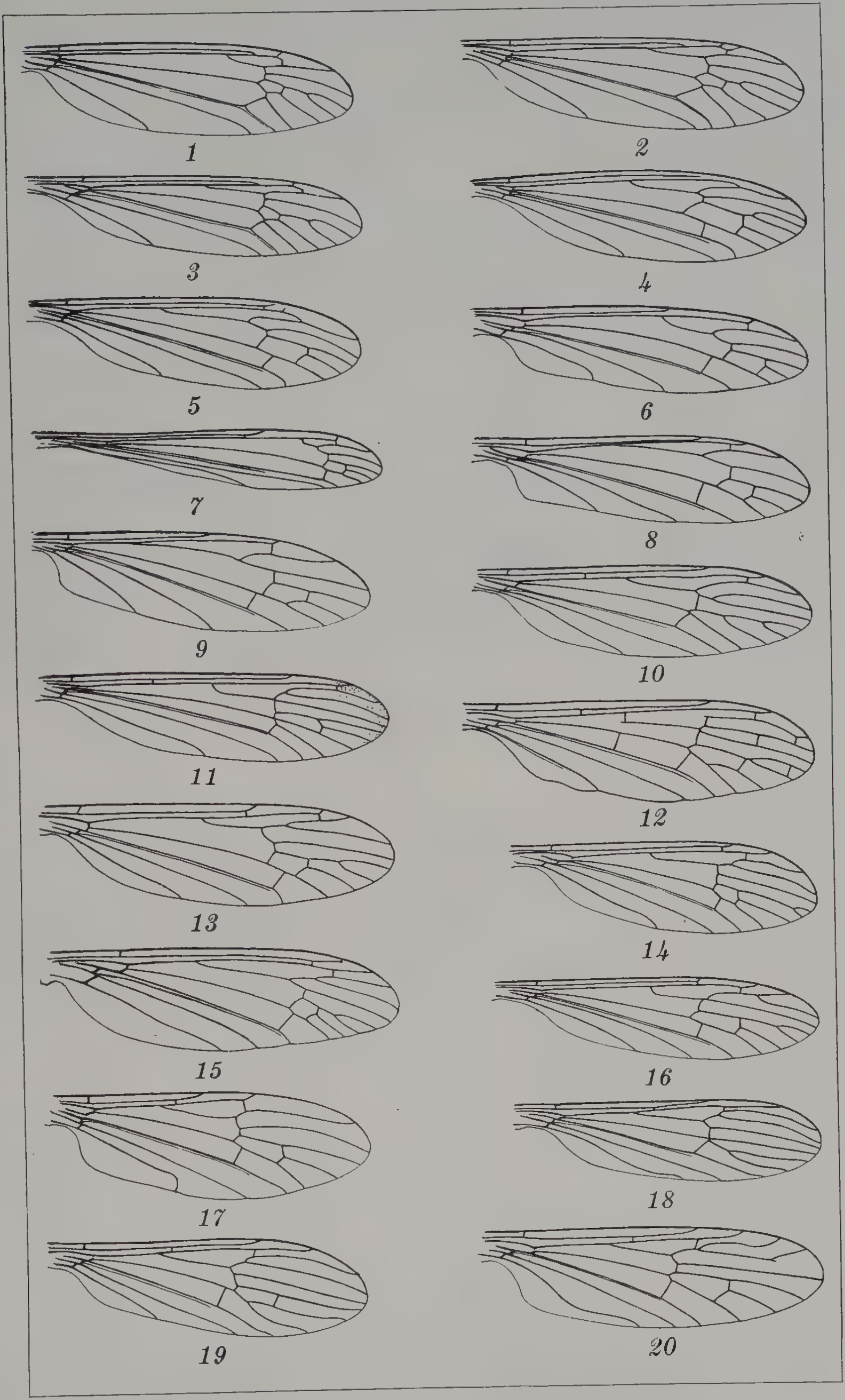
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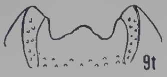
PLATE 1.



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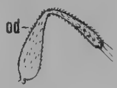

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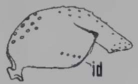

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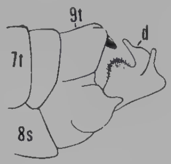

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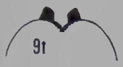

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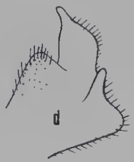

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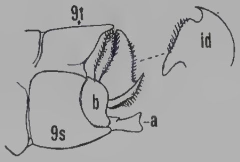

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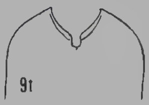

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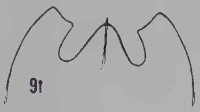

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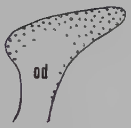

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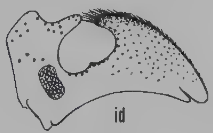

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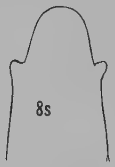

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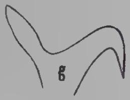

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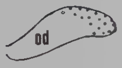

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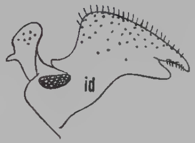

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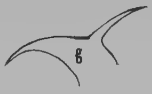

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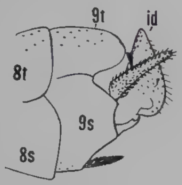
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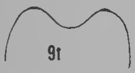

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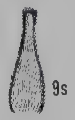

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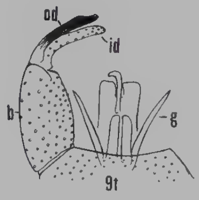

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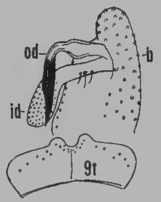

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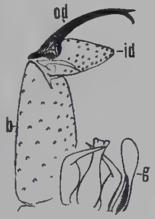

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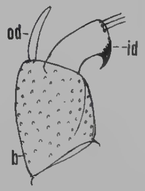

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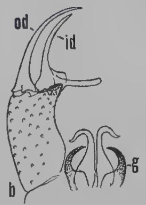

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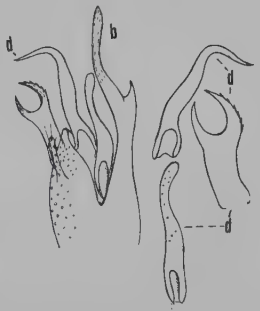

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PLATE 2.

THE PHILIPPINE
JOURNAL OF SCIENCE

VOL. 44 APRIL, 1931 NO. 4

NEW OR NOTEWORTHY PHILIPPINE ORCHIDS, I

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and

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SIXTEEN PLATES

Incidental to the preparation of a popular treatise on the orchids of the Philippines, the junior author has encountered several new species and varieties, descriptions of which seemed best to publish in a separate paper, together with notes on species that, though represented in other parts of the eastern Tropics, have not heretofore been recorded as natives of the Philippines. As a popular book is hardly the proper medium for the announcement of scientific discoveries, it is planned to publish in the Philippine Journal of Science a series of papers dealing with the new orchids now in hand and with the new or noteworthy species that are constantly being discovered through intensive exploration. Of the proposed series the present paper is the first number.

With very few exceptions, every Philippine orchid that has been illustrated in scientific publications in the last quarter of a century has been reconstructed from dead material in which the delicate structures of the flowers had been altered by desiccation. In the present series of papers it is proposed to issue plates that will portray the species discussed from living plants and will clearly represent diagnostic structures.

With the increasing interest that is being taken in the orchids of the Philippines by owners of gardens in the vicinity of Manila, there has developed a pressing need for critical studies of the horticultural forms which are commanding attention. The time has come when the study of living material should be undertaken more and more intensively, not only to solve horticultural problems, but to verify and amplify the results of the investigations through which our present knowledge of the Philippine orchid flora has been gained.

The types of the new species and varieties herein described have been deposited in the herbarium of the Bureau of Science and are represented by isotypes in the herbarium of Oakes Ames. Available isotypes will be distributed to American and European herbaria. All of our descriptions in the following pages have been prepared from living material with the exception of the one devoted to *Bulbophyllum ornatissimum*. All illustrations are by Mr. José M. Salazar, draftsman of the division of botany, Bureau of Science. The color terms used are mostly from Ridgway's Color Standards and Color Nomenclature (1912).

The present paper contains the descriptions of four species and one variety that appear to be new to science. Several previously described species are also included, together with notes and descriptions. *Haemaria* is included as a genus new to the Philippines.

The authors are under obligation to Dr. William H. Brown, Director of the Bureau of Science, who has given enthusiastic support and encouragement to the study of Philippine orchids.

Genus ADENOSTYLIS Blume

ADENOSTYLIS MERRILLII sp. nov. Plates 1, 3, and 7.

Affinis *Adenostylis luzonensis* Ames. Caules teretes, erecti, 10 ad 14 cm alti, 3 ad 4 mm in diametro, succulenti, supra folia longe villosi, pilis albidis. Folia prope medium caulium conferta, basibus petiolorum vaginantibus; lamina ovata ad ovato-lanceolata, acuta, plus minusve obliqua, 2.5 ad 4.5 cm longa, 1.4 ad 2.7 cm lata, membranacea. Bracteae inflorescentiae membranaceae, anguste lanceolatae, longe acute acuminatae, 8 ad 18 mm longae, sparsim pubescentes. Racemi 7.5 ad 8 cm longi, villosi. Flores in racemo laxo dispositi, albi, pedicello cum ovario dense villoso, cylindrico, anguste obovoideo, circiter 10 mm longo. Sepalum dorsale concavum, ovatum, circiter 9 mm longum, 5 mm latum, 5-nervium, extus villosum, petalis adhaerens. Sepala lateralia extus pubescentia, oblongo-lanceolata, obtusa, circiter 9 mm longa, 3 mm lata, 3-nervia. Petala obliquissime oblanceolata, glabra, alba, obtusa, 3-nervia. Labellum cuneato-oblanceolatum, circiter 9.5 mm longum, 5 mm latum, concavum, prope apicem leviter trilobatum. Columna brevis, circiter 6 mm longa; rostellum erectum, bipartitum. Pollinia globosa, caudicula gracili affixa; glandula oblonga, alba.

Terrestrial, succulent herb. Stems terete, erect, sometimes with decumbent base, 10 to 14 cm high, 3 to 4 mm in diameter, long villose above the leaves, the hairs white. Leaves crowded above the middle of the stem with bases vaginate, the lamina ovate to ovate-lanceolate, acute, somewhat asymmetrical, 2.5 to 4.5 cm long, 1.4 to 2.7 cm wide, membranaceous, light green, reticulations distinct dark green. Bracts membranaceous, narrowly lanceolate, long and acutely acuminate, 8 to 18 mm long, sparsely pubescent. Racemes 7.5 to 8 cm long, villose. Flowers laxly arranged, white; the pedicel and ovary villose, cylindric; narrowly obovoid, about 10 mm long, grass green to kildare green. Dorsal sepal concave, ovate, buff-pink, about 9 mm long, 5 mm wide, villose on the outside, 5-nerved, the petals adhering. Lateral sepals white, buff-pink at the apex, the central nerve greenish, villose outside, oblong-lanceolate, obtuse, about 9 mm long, 3 mm wide, 3-nerved. Petals white, glabrous, obtuse, 3-nerved. Labellum white with a coral red line at the center and greenish at the base, cuneate-oblanceolate, about 9.5 mm long, 5 mm wide, concave, slightly trilobed near the apex. Column short, about 6 mm long; the rostellum erect, bipartite. Pollinia globose, the glands oblong, white.

LUZON, Manila, Bureau of Science orchid house, *Bur. Sci. 78918 Quisumbing,* March 10, 1930. The living plants from Baguio, Mountain Province, were collected by Mrs. K. B. Day, who found them growing on moss-covered decayed logs and in mossy ground, at about 1,600 meters altitude.

This species is closely allied to *Adenostylis luzonensis* Ames, from which species it differs conspicuously in its subentire labellum and lower stature.

Genus HAEMARIA Lindley

HAEMARIA DISCOLOR Lindl. Plates 1 and 8.

Haemaria discolor LINDL., Gen. and Spec. Orch. (1840) 490; SCHLECHTER, Die Orchideen (1927) 118, *fig. 22.*

Goodycra discolor KER in Bot. Reg. (1818) *t. 271;* Lodd. Bot. Cab. (1817?) 148, Bot. Mag. (1819) *t. 2055.*

Ludisia discolor A. RICH. in Blume Fl. Jav. Orch. (1858) 95, *t. 42, fig. D.*
Ludisia furetii BLUME, Fl. Jav. Orch. (1858) 96, *t. 34, fig. 3.*
Ludisia odorata BLUME, Fl. Jav. Orch. (1858) 95, *t. 34, fig. 2.*

Stems succulent, naked, procumbent, glabrous below the leaves, brownish purple, ascending, 7 to 10 mm in diameter. Leaves alternate, approximate, ovate to elliptic, acute or apiculate, 3.5 to 7.5 cm long, 2 to 3.5 cm wide, the petioled base vaginate, upper surface velvety green to nearly black, with five prominent coral red lateral nerves (sometimes the venation reticulated; reticulations when present coral red), lower surface 3-nerved, the nerves vernonia-purple. Scape erect, terete, pale green, pubescent, 15 to 20 cm long; the inflorescence few-flowered; the bracts broadly lanceolate, shorter than the ovary. Flowers sessile, white except the sulphur yellow gynostemium, loosely arranged, odorless, 17 to 19 mm across. Ovary somewhat spirally twisted, white-pubescent, striped with green, about 15 mm long. Dorsal sepal cucullate, broadly ovate, obtuse, about 9 mm long, 7 mm wide. Lateral sepals slightly twisted, similar to the dorsal sepal. Petals lanceolate-linear, obtuse, adherent to the dorsal sepal. Labellum unguiculate, twisted, abruptly 2-lobed at the apex, lobes oblong, spreading. Gynostemium clavate, twisted. Anther horizontal, ovoid, acuminate, point slightly twisted; locules 2, parallel. Pollinia 2, clavate.

LUZON, Manila, Bureau of Science orchid house, *Bur. Sci. 78902 Quisumbing*, February 20, 1930. Cultivated in Mrs. George Fairchild's gardens at Pasay, Rizal Province. Of Philippine origin, but the locality and province unknown. This orchid is often classified among the "jewel orchids," and is easily cultivated in pots, and reproduced by cuttings of the stem. It needs plenty of water. The plants flower during January and February.

This species has not heretofore been recorded from the Philippines, although it is known to be a native of the Malay Peninsula and China. According to the shape and color of the leaves several varieties were recognized.

Genus DENDROBIUM Swartz

DENDROBIUM CHAMELEON Ames. var. **OVATILABIUM** var. nov. Plates 1, 5, and 9.

A specie labelli lamina ovata solum differt.

LUZON, Manila, Bureau of Science orchid house, *Bur. Sci. 77227 Quisumbing*, August 12, 1929. The living specimens collected in Floridablanca, Pampanga Province, hanging on trees, at medium altitudes. The leaves appear about December.

This variety differs conspicuously from *Dendrobium chameleon* Ames in its ovate labellum and in the color of its flowers.

Forma ALBA forma nov.

Species similis differt floribus colorem.

LUZON, Manila, Bureau of Science orchid house, *Bur. Sci. 77227a Quisumbing,* August 16, 1929. Living plants from Floridablanca, Pampanga Province, hanging on trees, at medium altitudes.

This form is identical with the species in every respect except that the flowers are pure white with a cream-colored spur.

DENDROBIUM MIYASAKII sp. nov. Plates 1, 4, and 9.

Species *D. victoriae-reginae* Loher affinis, floribus multo majoribus differt. Caules sulcati, pauci et gracile, superne pauciramosi, usque ad 75 cm alti, 4 mm in diametro, internodia plerumque 1 ad 4.5 cm longa. Folia lineari-lanceolata, acuta, 6 ad 8.5 cm longa, 0.5 ad 1.1 cm lata, subcoriacea, in basi amplectentia. Pedunculus circiter 5 mm longus. Flores singuli, 4 ad 5.5 cm longi, 4 ad 5.5 cm lati; ovarium cum pedicello tenue, 2.5 ad 3 cm longum. Sepalum dorsale oblongo-lanceolatum, obtusum, 2.3 ad 2.5 cm longum, 0.9 ad 1.1 cm latum. Sepala lateralia triangulari-lanceolata, obtusa, in mentum obtusum producta, 4.2 ad 4.5 cm longa, 1.3 ad 1.4 cm lata; mentum 2.1 ad 2.4 cm longum. Petala elliptica vel elliptico-ovata, obtusa vel rotundata vel leviter retusa, 2.1 ad 3 cm longa, 1.4 ad 1.8 cm lata, margine anteriore minutissime crenulato. Labellum simplex, breviter unguiculatum, e basi cuneata, obovatum vel oblanceolatum, 3.5 ad 4.3 cm longum, antice dilatatum, 1.6 ad 2.5 cm latum, in apice retusum. Gynostemium lobulatum.

Stems erect, sulcate, few and slender, up to 75 cm high, 4 mm in diameter, dark purple; branches few, rooting at the nodes, greenish, about 2 mm in diameter. Leaves linear-lanceolate, acute, 6 to 8.5 cm long, 0.5 to 1.1 cm wide, the uppermost ones about 2.5 cm long, subcoriaceous when dry, clasping at base. Peduncles about 5 mm long. Flowers solitary at the end of the branches, pendulous, not fragrant, 4 to 5.5 cm long, 4 to 5.5 cm across; pedicellate ovary slender, 2.5 to 3 cm long. Sepals white, suffused with mallow pink half way to the apex. Dorsal sepal oblong-lanceolate, obtuse, 2.3 to 2.5 cm long, 0.9 to 1.1 cm wide. Lateral sepals triangular-lanceolate, obtuse, with the spur

4.2 to 4.5 cm long, 1.3 to 1.4 cm wide; spur 2.1 to 2.4 cm long, white with obtuse grass green tip. Petals elliptic to elliptic-ovate, obtuse, rounded or somewhat retuse, 2.1 to 3 cm long, 1.4 to 1.8 cm wide, the anterior margins minutely crenulate, mallow pink to light mallow purple, white at the base. Labellum simple, obovate to oblanceolate, somewhat unguiculate, narrowed at the cuneate base, 3.5 to 4.3 cm long, 1.6 to 2.5 cm wide, the apex retuse, the expanded portion minutely crenulate, mallow purple, with three true purple lines extending on the narrowed portion to the base. Gynostemium lobulate, white, with purple markings on the margins.

LUZON, Manila, Bureau of Science orchid house, *Bur. Sci. 78823 Quisumbing*, December 29, 1929. The living specimens collected from Pampanga Province, on rocks on the hills back of Floridablanca. Flowers during December to March.

A previous collection referable to this species, was made in Zambales Province, on rocks of Mount Tapolao at 2,000 feet altitude, November-December, 1924, *Bur. Sci. 44715 Ramos and Edaño*. The flowers were described by the collectors as being pink and white.

A species closely allied to *Dendrobium victoriae-reginae* Loher but differing radically in its habit and in its much larger flowers. The species is dedicated to Mr. S. Miyasaki, enthusiastic orchid grower in Manila, in whose garden the junior author has enjoyed every facility for the study of orchids and to whom he is under many obligations.

Genus ERIA Lindley

ERIA APOROIDES Lindl.

Dendrobium aporoides (Lindl.) Merr. MERR., Sp. Blancoanae (1918) 113, Enum. Philip. Fl. Pl. 1 (1924) 345.

Upon critical examination of numerous living Philippine plants of this species it has been found that the pollen masses are eight in number. The species is therefore indubitably a member of the genus *Eria* as J. J. Smith has indicated, as a result of additional research, in Bull. Jard. Bot. Buit. Ser. 3, 8. Liv. 1 (1926) 49.

ERIA LONGISSIMA sp. nov. § Cylindrolobus. Plates 1, 5, 10, and 11.

Habitu *E. perspicabili* Ames similis. Caulis simplex, ad basi teres, superne leviter complanatus, foliosus, 50 ad 100 cm longus, 0.8 ad 1.3 cm in diametro, internodii 2 ad 3.5 cm longis. Folia lineari-lanceolata, glabra, in sicco subcoriacea, nervosa, attenuata, 9.5 ad 20 cm longa, 2 ad 4 cm lata, in apice inaequaliter

bilobulata, basi vaginanti. Inflorescentiae oppositifoliae, 2- ad 4-florae, cum pedunculo circiter 4.5 cm longae; pedunculus usque ad racemum brevis, circiter 1.5 cm longus. Bractae 5 vel 6, conspicuae, glabrae, oblongo-lanceolatae vel lanceolatae, subacutae, 19 ad 21 mm longae, 5 ad 7 mm latae, in basi cordatis. Flores circiter 1.5 cm longi; pedicellus glaber, cum ovario, 1.3 ad 2 cm longus. Sepalum dorsale oblongum, obtusum, circiter 15 mm longum, 6 mm latum. Sepala lateralia triangulari-lanceolata, obtusa, circiter 15 mm longa, 6.5 mm lata, subfalcata, mentum brevem formantia; mentum circiter 6 mm longum. Petala leviter oblique-oblonga, valde obtusa, circiter 13 mm longa, 5 mm lata, membranacea, marginibus aliquando inaequaliter et leviter irregularibus. Labellum in circuitu suborbiculare, prope apicem 3-lobatum, circiter 9 mm longum, in basi breviter unguiculatum; lobi laterales breves, obtusi, circiter 1.5 mm longi; lobus medius late ovatus, obtusus, incrassatus, circiter 3 mm longus, margine subcrispato. Discus per medium carinis 3 humilibus percursus quae in lobo medio confluunt, carinis in basi utrinque dilatatis. Gynostemium circiter 8 mm longum, crassum, apice bilobatum, in pedem 3 mm longum productum.

An epiphyte with much the habit of *Eria perspicabilis* Ames. Stems simple, terete at the base, somewhat complanate above, foliose, 50 to 100 cm long, 0.8 to 1.3 cm in diameter; the internodes 2 to 3.5 cm long. Leaves linear-lanceolate, glabrous, somewhat firm in living condition, subcoriaceous when dry, nervose, light green, 9.5 to 20 cm long, 2 to 4 cm wide, narrowed to the unequally bilobed apex, the base clasping and sheathed. Racemes opposite the leaves, 2- to 4-flowered, with the peduncle about 4.5 cm long; the peduncles up to the raceme about 1.5 cm long. Bracts citron yellow, 5 or 6, conspicuous, glabrous, oblong-lanceolate to lanceolate, obtuse, 19 to 21 mm long, 5 to 7 mm wide, the apex minutely apiculate, the base cordate. Flowers fragrant, recalling the odor of *Jasminum sambac*, conspicuous, white, about 1.5 cm long; the pedicels with the ovary glabrous, citron yellow, 1.3 to 2 cm long. Sepals and petals pure white with bases naphthalene yellow, the spur naphthalene yellow. Dorsal sepal oblong, obtuse, about 15 mm long, 6 mm wide. Lateral sepals triangular-lanceolate, obtuse, about 15 mm long, 6.5 mm wide, subfalcate, forming a rather short spur, which is about 6 mm long. Petals obliquely oblong or elliptic-oblong, broadly obtuse, about 13 mm long, 5 mm wide, membranaceous, margins sometimes slightly irregular. Labellum white without, cream-buff within, suborbicular in outline, 3-lobed near

the apex, about 9 mm long, shortly unguiculate at base; lateral lobes short, obtuse, about 1.5 mm long; middle lobe broadly ovate, obtuse, about 3 mm long, rather thickened, margin subcrispate. Disc traversed through the middle by 3 low keels which come together on the middle lobe, keels at base on each side dilated. Gynostemium about 8 mm long, rather thick, the apex bilobed, the foot about 3 mm long.

LUZON, Manila, Bureau of Science orchid house, *Bur. Sci. 77242 Quisumbing,* June 1, 1929. The living plants were collected from the mossy forests of Mount Maquiling, Laguna Province, Luzon.

This species has the appearance of *Eria perspicabilis* Ames but is most closely allied to *E. brachystachya* Reichb. f. and to *E. albolutea* Rolfe. It is larger throughout than *E. brachystachya* with a rather different lip. It has much larger leaves, fewer-flowered racemes, and apparently wider perianth parts than *E. albolutea* Rolfe.

Genus BULBOPHYLLUM Thouars

BULBOPHYLLUM BIFLORUM Teijsm. and Binn. Plates 2 and 12.

Bulbophyllum biflorum TEIJSM. and BINN., Nat. Tijdschr. Ned. Ind. 3 (1855) 397; J. J. SM., Fl. Buitenz. 6 (Orch. Jav.) (1905) 469, Fig.-Atlas (1911) *fig. 359;* MIQ., Fl. Ind. Bat. 3 (1859) 647; REICHB. f., Walp. Ann. Bot. Syst. 6 (1861) 262; NAVES, Novis. App. (1882) 234; AMES in Merr. Enum. Philip. Fl. Pl. 1 (1925) 390.

Cirrhopetalum biflorum J. J. SM., Ic. Bog. 2 (1903) 104, *t. 120 B;* SCHLTR., Die Orchideen (1927) 329; Sanders' Orchid Guide (1927) 113.

Phyllorchis biflora OK., Rev. Gen Pl. 2 (1891) 677.

Epiphyte. Pseudobulbs 4-angled, one-leafed, ovoid, 2.5 to 3.5 cm long, 1.3 to 2 cm wide. Leaves at the top of the pseudobulbs, oblong-elliptic, 7 to 11 cm long, 2.5 to 3.5 cm wide. Inflorescence arising from the base of the pseudobulb, two-flowered; the peduncle slender, about 8 cm long; bracts membranaceous. Flowers about 9.5 cm long, emit a peculiar odor, pedicel and ovary slender, about 1.5 cm long. Dorsal sepal elliptic with a filament, about 2.5 cm long, very pale pink to almost white striped and spotted with pomegranate purple, 5-nerved. Lateral sepals joined or free, greatly elongated into very slender filaments, 7.5 to 8 cm long, 0.6 to 0.65 mm wide, very pale pink to almost white, spotted and striped with pomegranate purple, the tips tawny purple, 5-nerved. Petals much smaller, about 6 mm long, 3.5 mm wide, with a minute apicule about 1 mm in length, 3-nerved. Labellum oblong-ovate, fleshy, recurved, yellowish brown, spotted with dark purple, about 5 mm long. Column somewhat fleshy, about 4 mm long, with the foot about 7 mm long.

LUZON, Manila, Bureau of Science orchid house, *Bur. Sci. 78844 Quisumbing*, May 28, 1929. Living plants collected from Mount Banahao, Tayabas Province, growing on tree trunks at medium altitudes. A native of Java.

A very interesting species characterized by its two-flowered inflorescences. Originally known from Java, now for the first time authentically reported from the Philippines.

BULBOPHYLLUM EMILIORUM sp. nov. Plates 2, 6, and 13.

Affine *B. copelandii* Ames. Rhizoma repens, circiter 5 mm in diametro, radicibus numerosis simplicibus vel ramosis. Pseudobulbi 1.8 ad 2 cm longi, 1.2 ad 1.3 cm in diametro, monophylli, 8 ad 10 cm distantes. Folia elliptico-oblonga, obtusa, coriacea, rigida, 19 ad 21 cm longa, 6 ad 6.5 cm lata. Pedunculus gracilis, circiter 1 cm longus, florem singulum speciosum gerens, pedicello cum ovario 4.5 ad 5.5 cm longo, bracteis membranaceis. Sepalum dorsale oblongo-lanceolatum, tenuiter acute acuminatum, circiter 3.5 cm longum, 1.2 cm latum. Sepala lateralia sigmoideo-lanceolata, tenuiter acuminata, circiter 3.2 cm longa, prope basim 1.3 cm lata. Petala anguste lanceolata, acuminata apice recurvata, fastigiata, circiter 3 cm longa, 0.8 cm lata. Labellum cum pede elongato columnae articulatum, recurvatum, carnosum; lobi laterales breves, rotundati, obtusi, circiter 2 mm longi; lobus medius lineari-lanceolatus, acutus, circiter 8 mm longus, 3.5 mm latus, recurvatus, carnosus, rigidus. Columna crassa, brevissima, erecta, circiter 4 mm longa et lata, basis lata in pedem 9 mm longum transiens.

Rhizomes creeping, about 5 mm in diameter; roots numerous, simple or branching. Pseudobulbs 1.8 to 2 cm long, 1.2 to 1.3 cm in diameter, monophyllous, 8 to 10 cm distant. Leaves elliptic-oblong, obtuse, coriaceous when dry, rather thick when fresh, rigid, 19 to 21 cm long, 6 to 6.5 cm wide. Inflorescence one-flowered; the peduncles slender, rather short, about 1 cm long; pedicel and ovary 4.5 to 5.5 cm long; bracts membranaceous. Dorsal sepal oblong-lanceolate, slenderly and acutely acuminate, about 3.5 cm long, 1.2 cm wide. Lateral sepals sigmoid-lanceolate, slenderly acuminate, about 3.2 cm long, 1.3 cm wide near the base. Petals narrowly lanceolate, acuminate with recurved apex, fastigiate, about 3 cm long, 0.8 cm wide. Lip hanging on a very slender foot; lateral lobes short, rounded, spreading, obtuse, about 2 mm long; middle lobe linear-lanceolate, acute, about 8 mm long, 3.5 mm wide, recurved, fleshy, rigid. Column thickened, very short, erect, about 4 mm long and broad, the foot about 9 mm long. Pollinia triangular.

MINDANAO, Agusan Province, Butuan, Barrio Guadalupe, *Emilio Quisumbing, s. n., July 1929*, on tree trunks in thick forests at low altitudes. Flowers fragrant, recalling the odor of *Dendrobium anosmum* Lindl. Peduncles pinard yellow; lateral sepals and dorsal sepal wax yellow on the back, mottled with amaranth purple, the apices suffused with bordeaux; the bases of the dorsal sepal and petals suffused with amaranth purple within; the petals white on the back; lip bordeaux half-way to the apex and pinard yellow at the base; column and foot pinard yellow.

A species closely allied to *Bulbophyllum copelandii* Ames, differing in its much larger leaves and flowers and in its differently shaped dorsal and lateral sepals, petals and labellum. The species is dedicated to Mr. Emilio Quisumbing, civil engineer, who collected the living specimens.

BULBOPHYLLUM ANTENNIFERUM (Lindl.) Reichb. f. Plates 2 and 14.

Bulbophyllum antenniferum (Lindl.) Reichb. f. in (Ames) Merr. Enum. Philip. Fl. Pl. 1 (1925) 379.

Rhizome creeping, 4 to 5 mm in diameter. Pseudobulbs one-leafed, 4-angled, ovoid, 1.5 to 2.5 cm long, 1.3 to 1.6 cm in diameter, 1 to 2 cm distant. Leaves rather thick, oblanceolate, elliptic-lanceolate or oblong-oblanceolate, rounded at the apex, subretuse, 8 to 15 cm long, 3 to 4.8 cm wide. Inflorescences usually one-flowered, very seldom two-flowered, conspicuous; peduncles 9 to 13.5 cm long, green. Bracts sheathing, about 1.5 cm long. Pedicel and ovary 4 to 5 cm long. Dorsal sepal large, ovate-lanceolate to lanceolate, somewhat fleshy, 9-nerved, carinate, 4 to 6.5 cm long, 1.2 to 1.5 cm wide, corinthian purple on the back, reed yellow in front, suffused with some corinthian purple and spotted with transparent white. Lateral sepals connate, smaller than the dorsal sepal, individually 6- or 7-nerved, narrowly lanceolate, falcate, 3.5 to 5 cm long, 0.7 to 0.8 cm wide, citron green and corinthian purple at the base. Petals very much smaller than the sepals, ovate-lanceolate, with prolonged filiform apex, terminating in a pyriform tip, pale grass green, striped with light purple, 3-nerved, 12 to 13 mm long, 2.5 to 3.5 mm wide. Lip recurved, fleshy, pilose above, corinthian purple at the apex, 4 to 5 mm long. Gynostemium 3-toothed, about 3 mm long. Pollinia yellow, somewhat ovoid.

LUZON, Manila, Bureau of Science orchid house, *Bur. Sci. 78847 Quisumbing,* September 24, 1929, living plants collected in Paete, Laguna Province; *Bur. Sci. 78845 Quisumbing,* December 11, 1929, living plants collected on Mount Mariveles, Bataan Province, creeping on tree trunks at low and medium altitudes.

BULBOPHYLLUM EBRACTEOLATUM Kränzl. Plates 2, 3, and 15.

Bulbophyllum ebracteolatum KRÄNZL. in Ann. k. k. Nat. Hofmus. Wien 30 (1916) 58; AMES in Merr. Enum. Philip. Fl. Pl. 1 (1925) 382.

Rhizomes creeping, 3.5 to 7 mm in diameter. Pseudobulbs one-leafed, remote, 4- or 5-angled, ovoid to ovoid-lanceolate, 3 to 4.5 cm long, 1.4 to 2 cm in diameter, 1.4 to 2 cm distant; the base of the pseudobulbs covered by two brown, membranaceous sheaths, 1.5 to 3.2 cm long, the lower one smaller. Leaves linear, 10 to 17 cm long, 1.7 to 2.5 cm wide, narrowed at both ends, the apex acute; petioles very short. Scape arising at the base of the pseudobulb, 5 to 8.5 cm long including the peduncles; the peduncles white flushed with some purple, pubescent with minute white hairs, 2 to 2.5 cm long, fleshy; the rachis fleshy, white, flushed with some purple, pubescent with minute white hairs, 5 to 6 mm in diameter. Basal sheaths two, brown, membranaceous, 8 to 10 mm long. Flowers odorless, minutely pubescent, 4.5 to 5 mm long, spirally arranged on the rachis, rather remote, the side view giving the appearance of a snake's head. Floral bracts covering the pedicels and ovaries cucullate, greenish or light purple, ovate, obtuse, 3 to 4 mm long. Pedicel and ovary very short, 1 to 2 mm long, purple, pubescent. Dorsal sepal cucullate, ovate, obtuse, 4 to 5 mm long, 2.5 to 3 mm wide, marguerite yellow to almost white, 3-nerved, the nerves pansy purple. Lateral sepals cucullate, connate, oblong, broadly obtuse to rounded, 4.5 to 5.5 mm long, 3 to 4 mm wide, marguerite yellow to almost white, 7-nerved, the nerves pansy purple. Petals very much smaller than the sepals, lanceolate, acutely acuminate, about 2 mm long, 1 mm wide, marguerite yellow to almost white, 1-nerved, the central nerve and smooth margins pansy purple. Lip fleshy, 3-lobed, about 3.5 mm long; the lateral lobes rather insconspicuous, minute, obtuse, up to 0.5 mm long, madder brown; the middle lobe oblong, rounded, lime green, pompeian red at the base. Foot slender, somewhat flattened, flesh color, about 1 mm long. Column white, fleshy, about 2 mm long, the apex pubescent, the arms two, bicuspidate. Pollinia ivory yellow, suborbicular.

LUZON, Manila, Bureau of Science orchid house, *Bur. Sci. 78924 Quisumbing,* February 27, 1930. Living plants collected from Mount Banahao, Laguna Province, as an epiphyte, at medium altitudes.

A species manifestly belonging in the group with *Bulbophyllum crassifolium* (Blume) J. J. Sm. and strongly characterized by its fleshy rachis, subsessile flowers, smooth-margined petals and by the bicuspidate wings or arms of the apex of the column.

BULBOPHYLLUM ORNATISSIMUM (Reichb. f.) J. J. Sm. Plate 16.

Bulbophyllum ornatissimum (Reichb. f.) J. J. SM. in Bull. Jard. Bot. Buit. 8 (1912) 26.

Cirrhopetalum ornatissimum REICHB. f. in Gard. Chron. 2 (1882) 424; KING and PRANTL, Ann. Roy. Bot. Gard. Calcutta 8 (1898) 95, *t. 133;* WARNER, Orch. Album 8 (1889) *t. 369;* HOOK. f., Fl. Brit. Ind. 5 (1890) 773; Sanders' Orch. Guide (1927) 116; SCHLECHT., Die Orchideen (1927) 331.

Creeping, the rhizome 2 to 2.5 mm in diameter. Pseudobulbs ellipsoid, 1.5 to 2 cm long, 2 to 3 cm distant, olive-green, bearing a single leathery leaf. Leaves oblong or elliptic-oblong, sessile, apex obtuse and slightly notched, 6 to 8 cm long, 1.3 to 1.7 cm wide. Scape one-flowered, arising from the base of the pseudobulb; peduncle about 10.5 cm long, bearing 2 or 3 sheathing bracts. Flower about 14 cm long. Pedicel and ovary about 13 mm long. Floral bract membranaceous, lanceolate, about 8.5 mm long. Dorsal sepal ovate, about 2 cm long, erect, 3-nerved, the apex extended into a long drooping filiform appendage bearing hairs on its edges in its lower part and paleæ in its apical portion. Lateral sepals lanceolate with filiform tails, about 12 cm long, the tips twisted, free at the base and apex, otherwise coherent. Petals lanceolate, falcate, about 1.5 cm long, 1-nerved, with filiform palea-bearing apices like the dorsal sepal. Lip oblong, obtuse, recurved from a very concave base, with three raised lines. Column broad with hooked decurved apical teeth which are dentate at their bases; foot curved, tapering. Anther pubescent in front. Pollinia attached to a viscous, oblong, compressed disc.

LUZON, Zambales Province, Mount Marayap, *Bur. Sci. 44806 Ramos and Edaño,* December 12, 1924, on tree trunks in the mossy forests, altitude about 400 meters. The flowers pale yellowish green with purple-brown markings, the paleæ and lip are crimson purple.

A unique species characterized by its greatly elongated lateral sepals and paleæ on the dorsal sepal and petals. A species otherwise known only from Assam and Sikkim.

ILLUSTRATIONS

PLATE 1

FIG. 1. *Dendrobium chameleon* Ames var. *ovatilabium* var. nov., side and front views of flower, × 1.
2. *Dendrobium chameleon* Ames forma *alba* forma nov., front view of flower, × 1.
3. *Haemaria discolor* Lindl., side view of flower, × 1.
4. *Haemaria discolor* Lindl., front view of flower, × 1.
5. *Dendrobium miyasakii* sp. nov., front view of flower, × 1.
6. *Dendrobium miyasakii* sp. nov., side view of flower, × 1.
7. *Adenostylis merrillii* sp. nov., side view of flower, × 2.
8. *Adenostylis merrillii* sp. nov., front view of flower, × 2.
9. *Eria longissima* sp. nov., portion of a flowering stem showing typical clusters of flowers, × 1.

PLATE 2

FIG. 1. *Bulbophyllum emiliorum* sp. nov., front view of flower, × 1.
2. *Bulbophyllum emiliorum* sp. nov., side view of flower, × 1.
3. *Bulbophyllum ebracteolatum* Kränzl., side view of flower, × 4.
4. *Bulbophyllum ebracteolatum* Kränzl., front view of flower, × 4.
5. *Bulbophyllum biflorum* Teijsm. and Binn., two flowers, × 1.
6. *Bulbophyllum antenniferum* (Lindl.) Reichb. f., front view of flower, × 1.
7. *Bulbophyllum antenniferum* (Lindl.) Reichb. f., side view of flower, × 1.

PLATE 3

ADENOSTYLIS MERRILLII SP. NOV.

FIG. 1. Petal, × 6.
2. Dorsal sepal and petals attached from back, × 6.
3. Lateral sepal from back, × 6.
4. Labellum, × 6.
5. Side view of column, × 6.

BULBOPHYLLUM EBRACTEOLATUM KRÄNZL.

FIG. 6. Habit, × 0.5.
7. Dorsal sepal, × 6.5.
8. Petal, × 6.5.
9. Lower view of confluent lateral sepals, × 6.5.
10. Upper view of confluent lateral sepals, column, and lip, × 6.5.
11. Upper view of column, × 7.5.
12. Side view of column, × 7.5.
13. Pollinia, × 25.
14. Labellum, × 7.5.

PLATE 4

DENDROBIUM MIYASAKII SP. NOV.

FIG. 1. Stem and branch, showing typical leaves, × 0.5.
2. Dorsal sepal, × 1.
3. Lateral sepal and spur, × 1.
4. Petal, × 1.
5. Labellum, × 1.
6. Front view of column, × 1.
7. Front and back views of anther cap, × 3.
8. Pollinia, × 5.

PLATE 5

DENDROBIUM CHAMELEON AMES VAR. OVATILABIUM VAR. NOV.

FIG. 1. Branch, × 0.5.
2. Portion of stems and flowers, approximately × 0.5.
3. Dorsal sepal, × 1.
4. Lateral sepal and spur, × 1.
5. Petal, × 1.
6. Labellum, × 1.
7. Front view of column, × 2.5.
8. Back view of anther cap, × 2.5.

ERIA LONGISSIMA SP. NOV.

9. Back and front views of a bract, × 1.
10. Dorsal sepal, × 1.
11. Lateral sepal, × 1.
12. Petal, × 1.
13. Side view of column, lip, pedicel, and ovary, × 2.
14. Front view of labellum, × 2.
15. Front view of column, × 2.
16. Back and front views of anther cap, × 7.

PLATE 6. BULBOPHYLLUM EMILIORUM SP. NOV.

FIG. 1. Habit, × 0.5.
2. Column, foot, and attachment of lateral sepal, × 3.25.
3. Petal, × 1.625.
4. Labellum from side, × 3.25.
5. Dorsal sepal, × 1.625.
6. Lateral sepal, front and back views, × 1.625.
7. Anther cap, × 6.5.
8. Pollinia, × 6.5.

PLATE 7

Adenostylis merrillii sp. nov.; habit, reduced.

PLATE 8

Haemaria discolor Lindl.; habit, reduced.

PLATE 9

FIG. 1. *Dendrobium chameleon* Ames var. *ovatilabium* var. nov.; habit, very much reduced.
2. *Dendrobium miyasakii* sp. nov.; habit, very much reduced.

Plate 10

Eria longissima sp. nov.; habit, very much reduced.

Plate 11

Eria longissima sp. nov.; flowering stem, natural size.

Plate 12

Bulbophyllum biflorum Teijsm. and Binn.; habit, reduced.

Plate 13

Bulbophyllum emiliorum sp. nov.; habit, reduced.

Plate 14

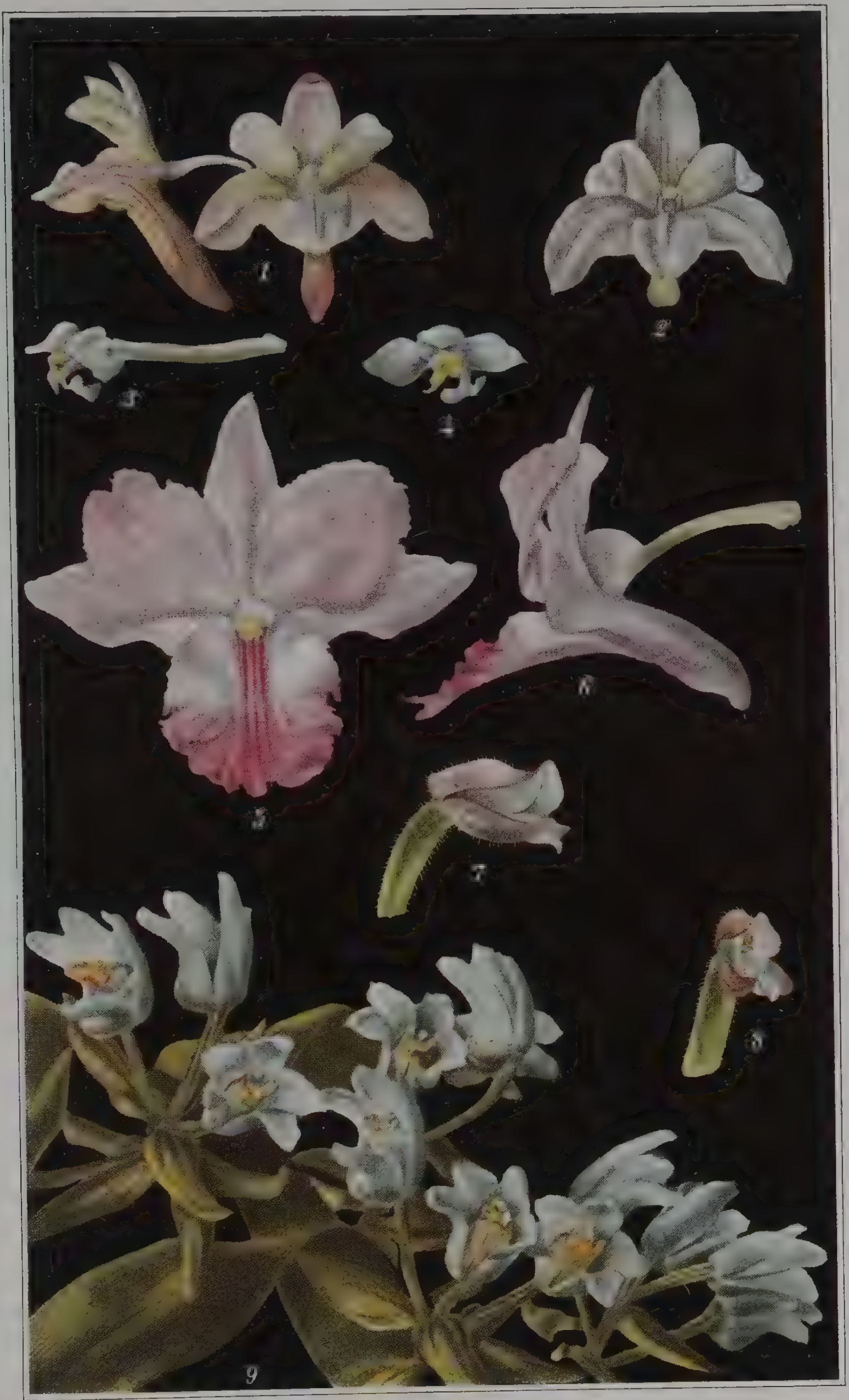
Bulbophyllum antenniferum (Lindl.) Reichb. f.; habit, reduced.

Plate 15

Fig. 1. *Bulbophyllum ebracteolatum* Kränzl.; habit, much reduced.
2. *Bulbophyllum ebracteolatum* Kränzl.; closer view showing scape and some pseudobulbs. Slightly reduced.

Plate 16

Bulbophyllum ornatissimum (Reichb. f.) J. J. Sm.; habit, reduced. (A photograph of the herbarium specimen.)



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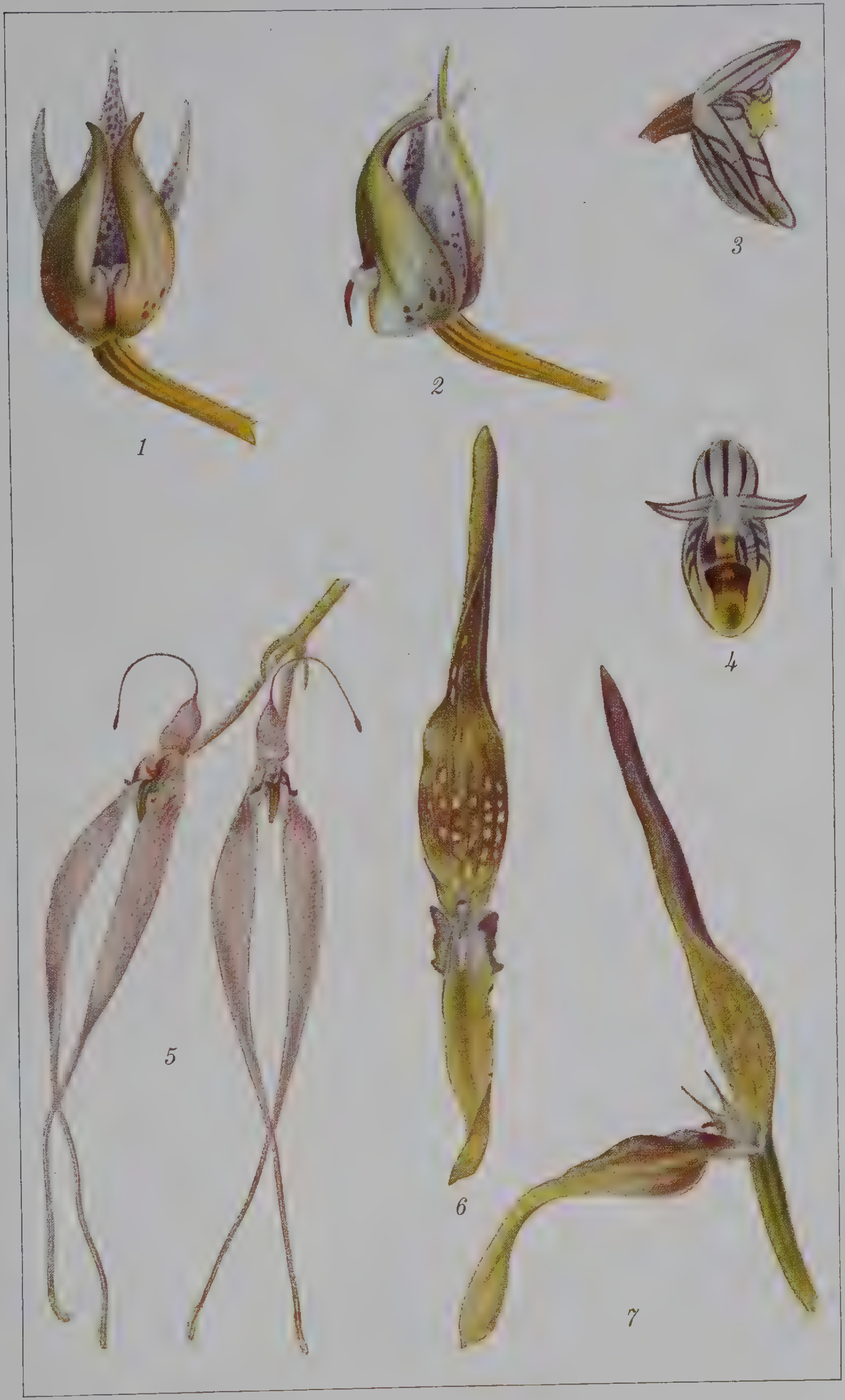

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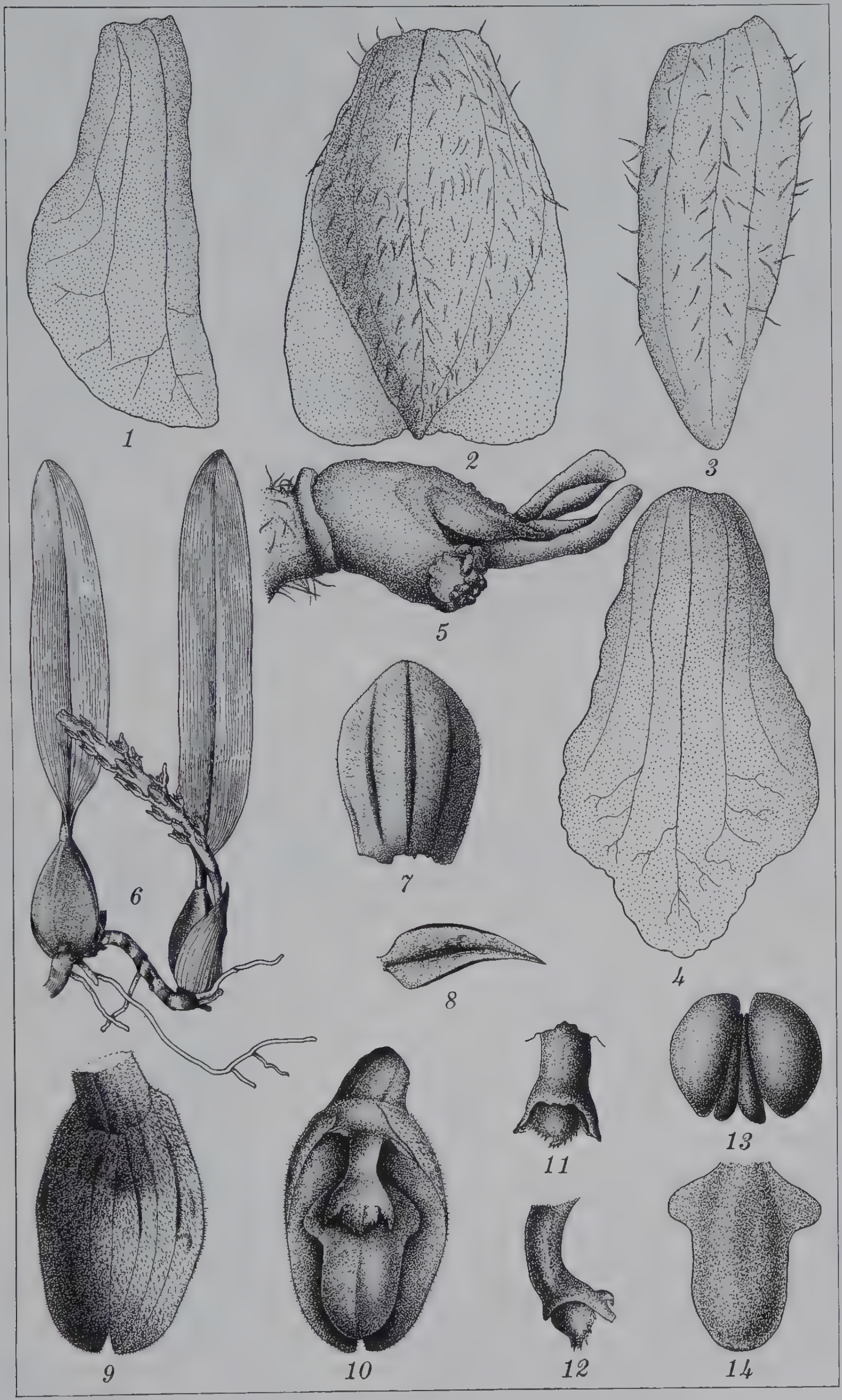
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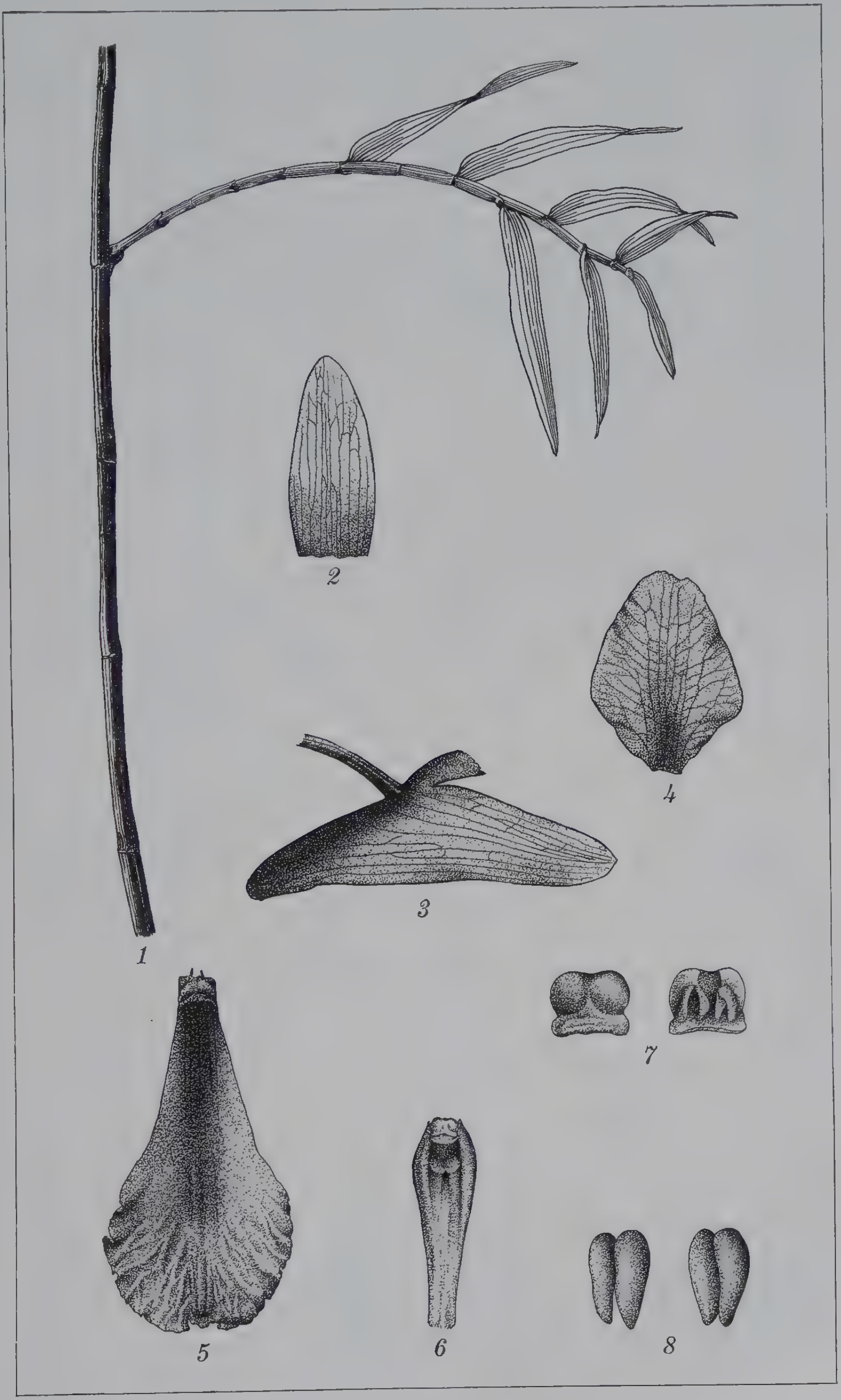

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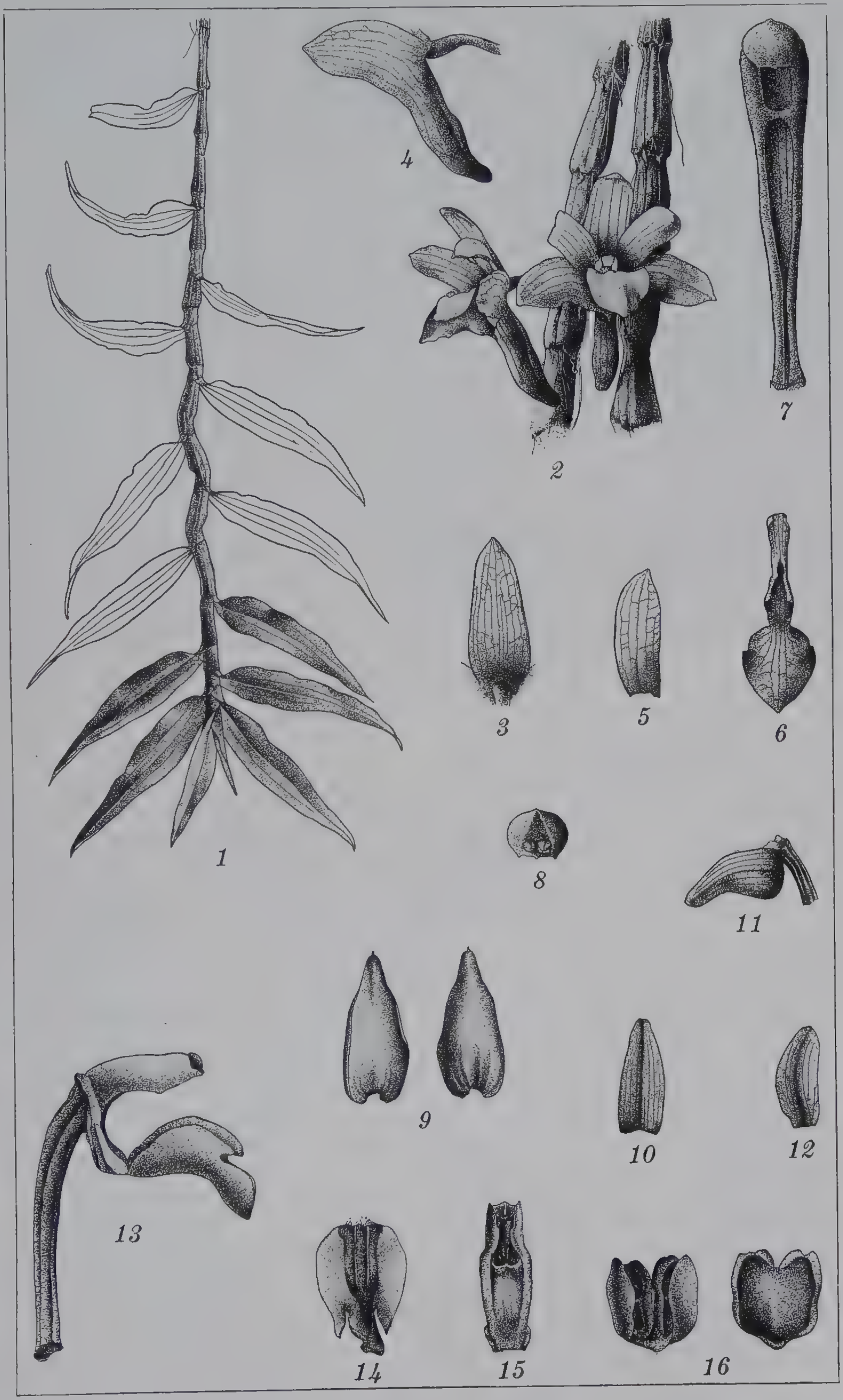
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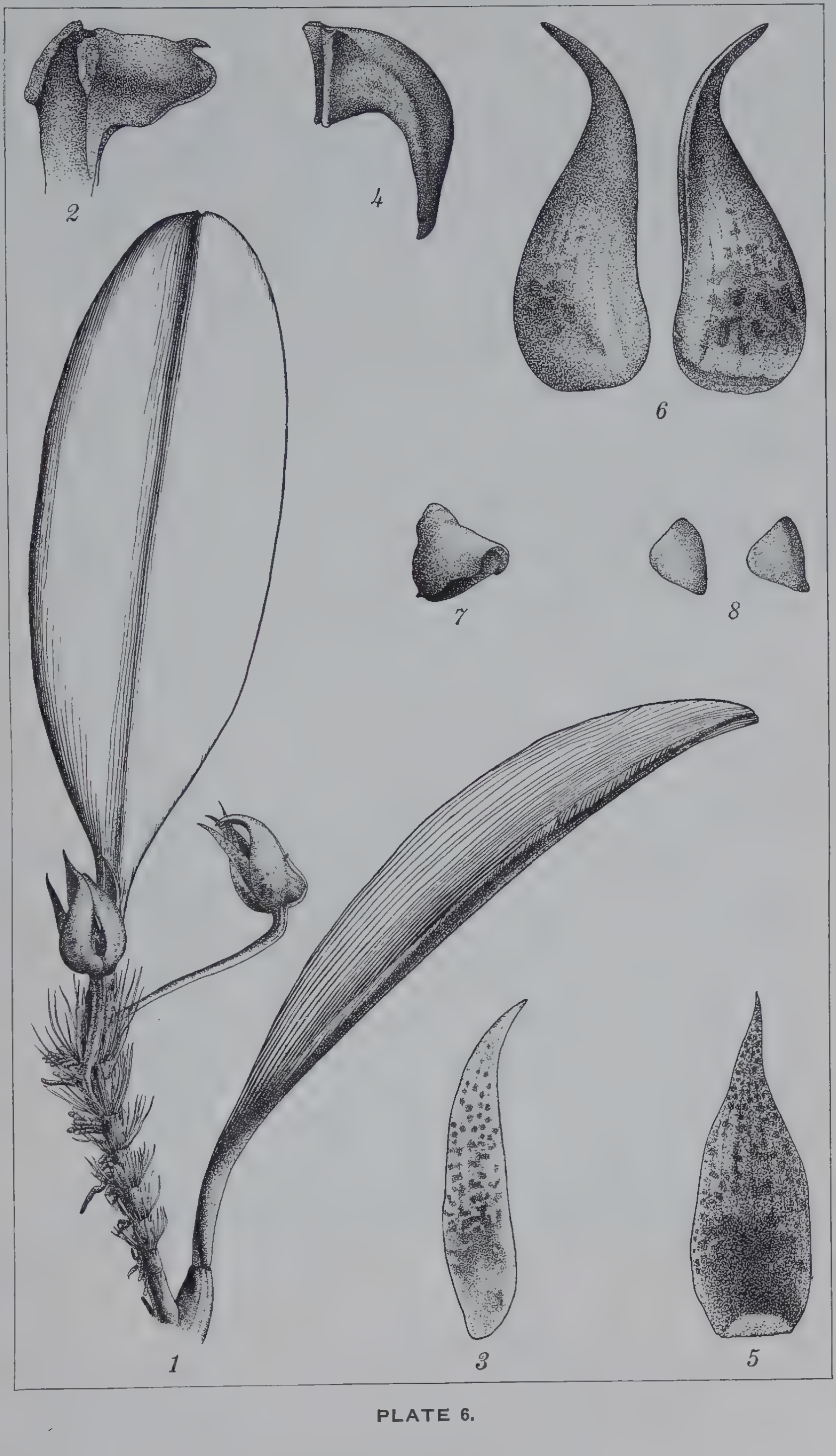
PLATE 5.



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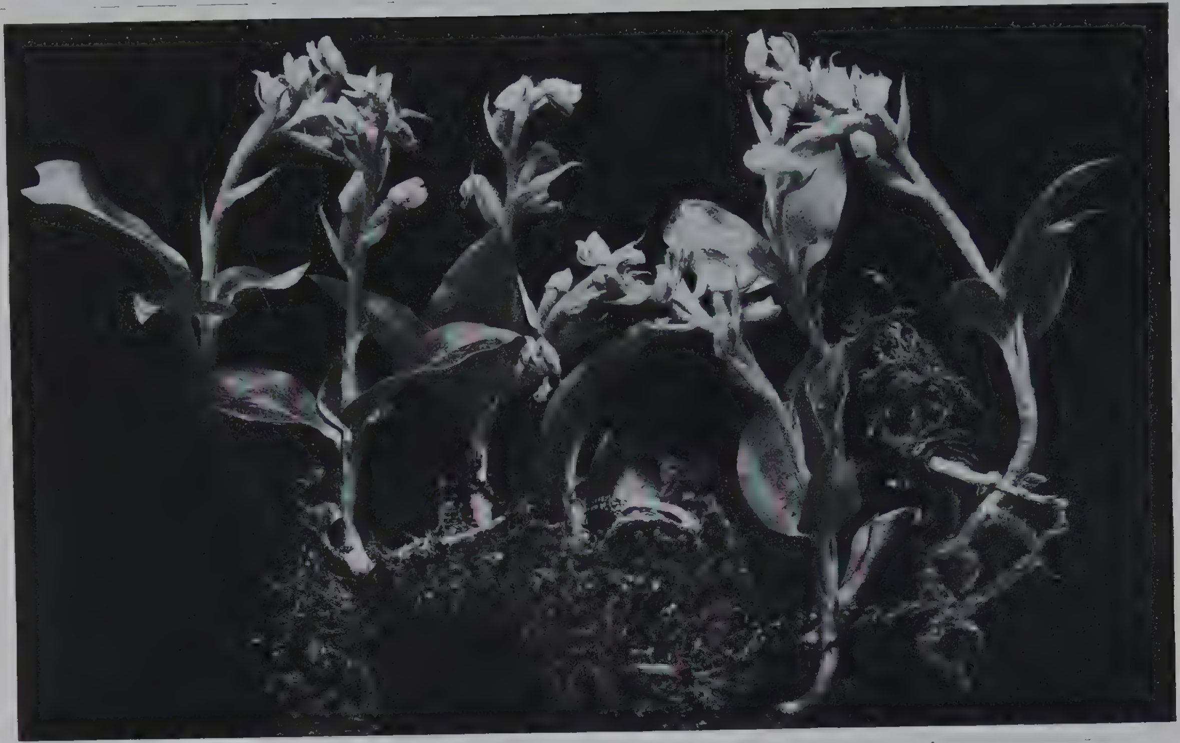

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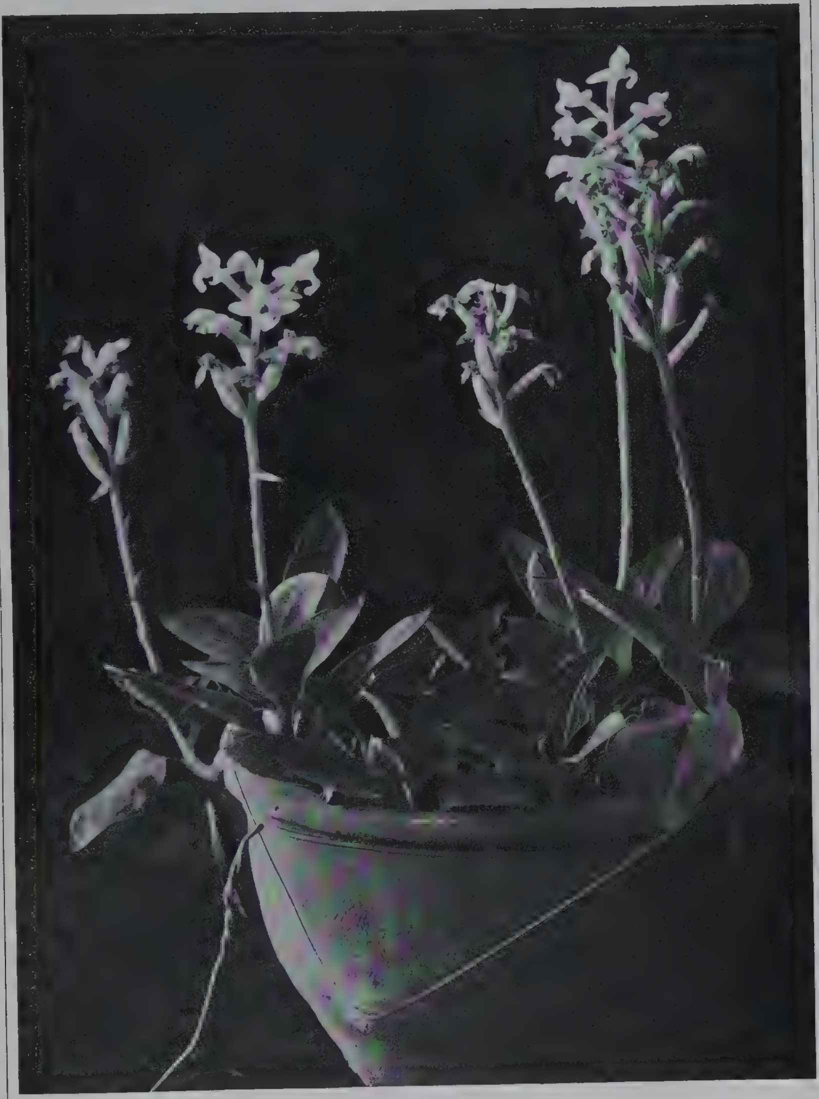

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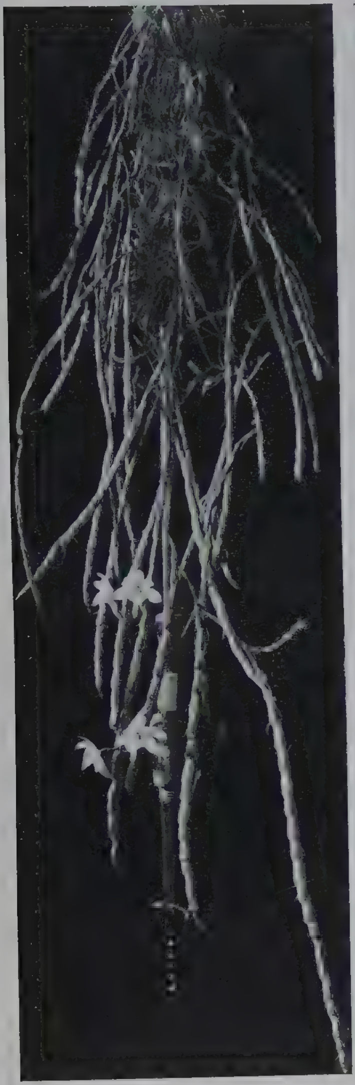

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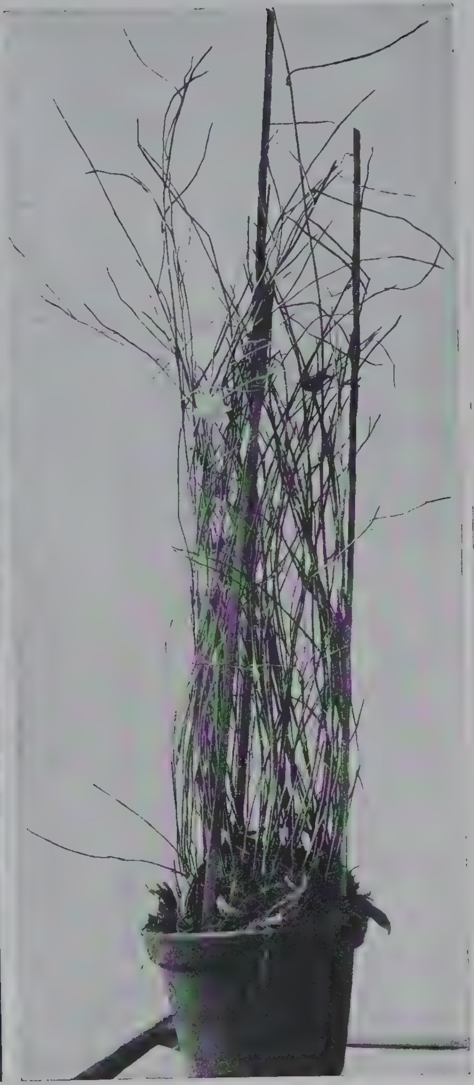

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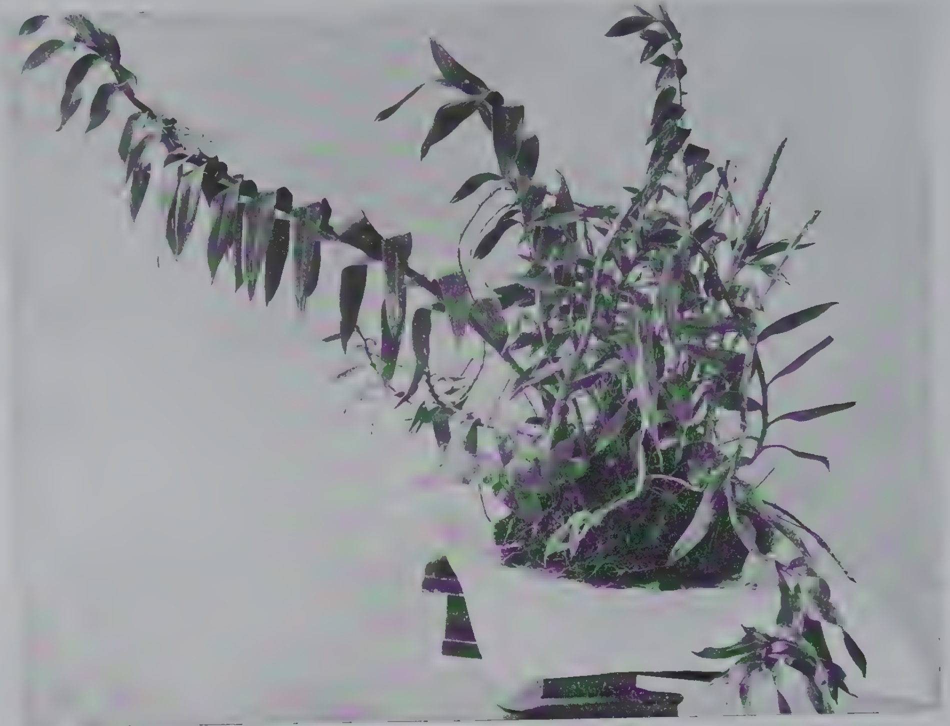

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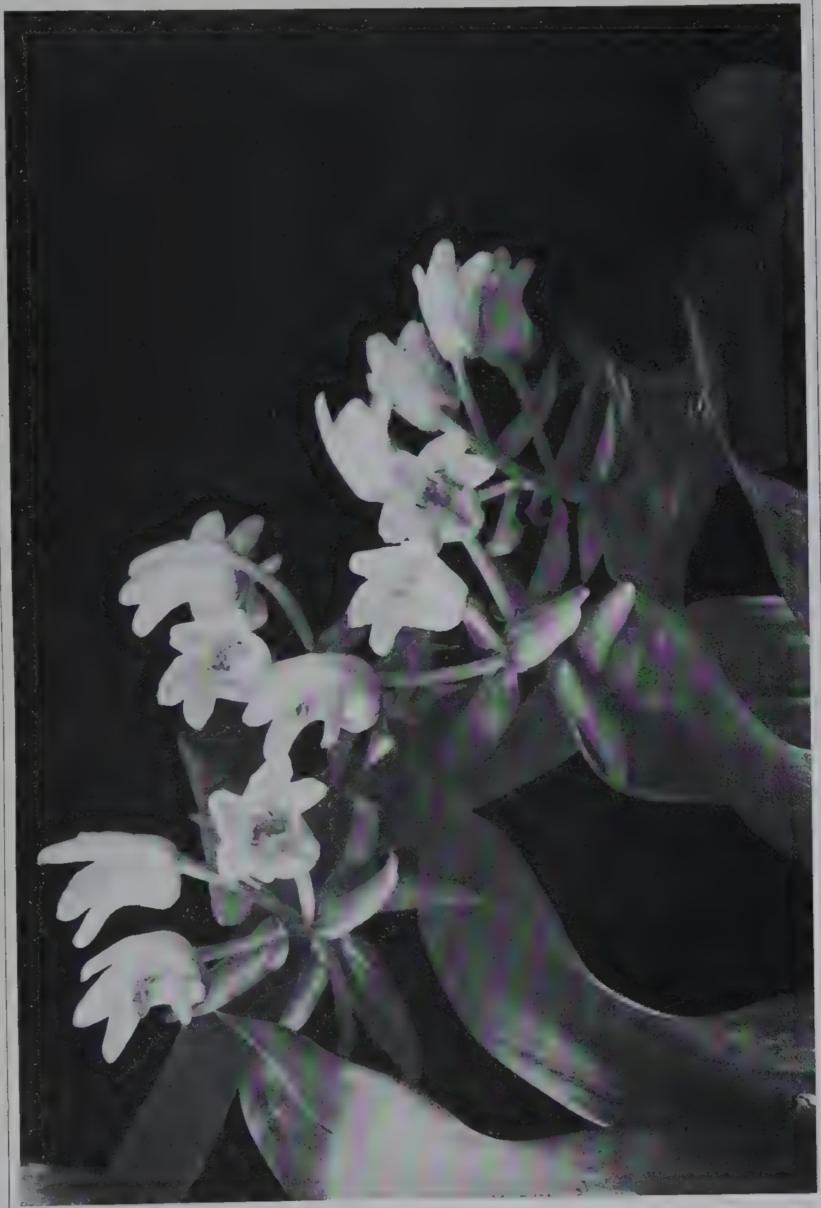

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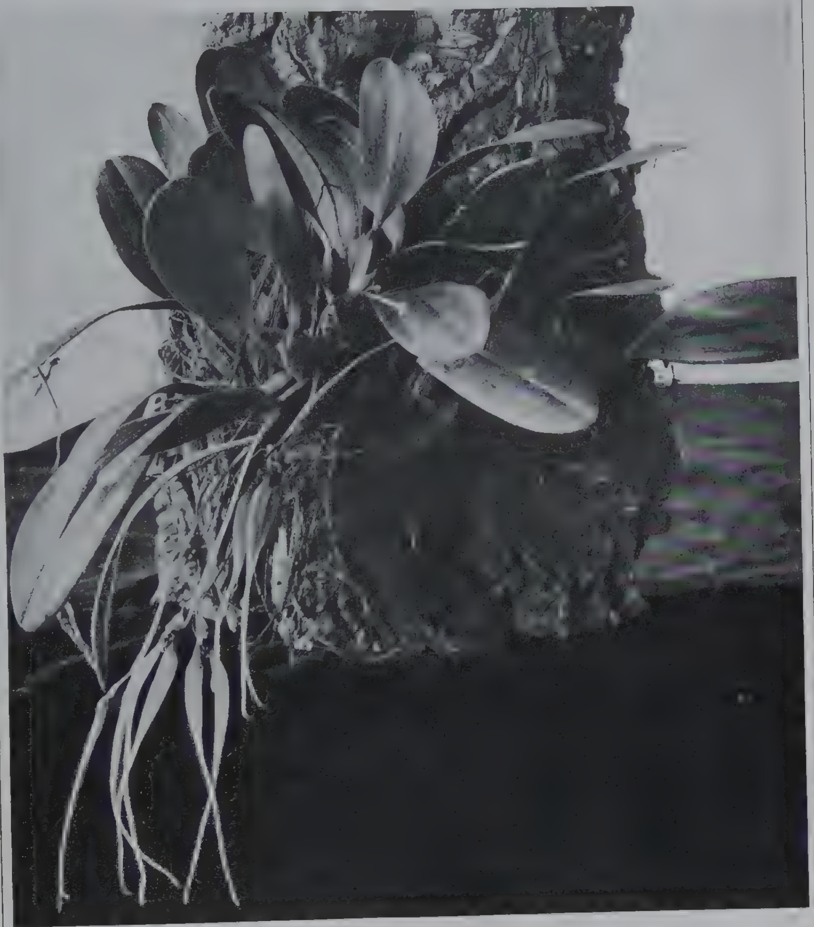

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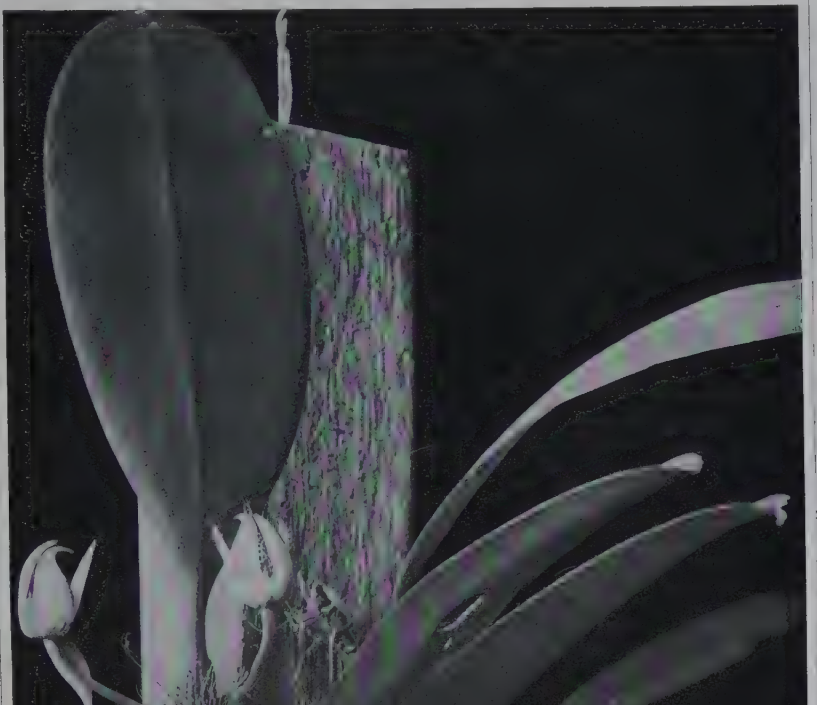

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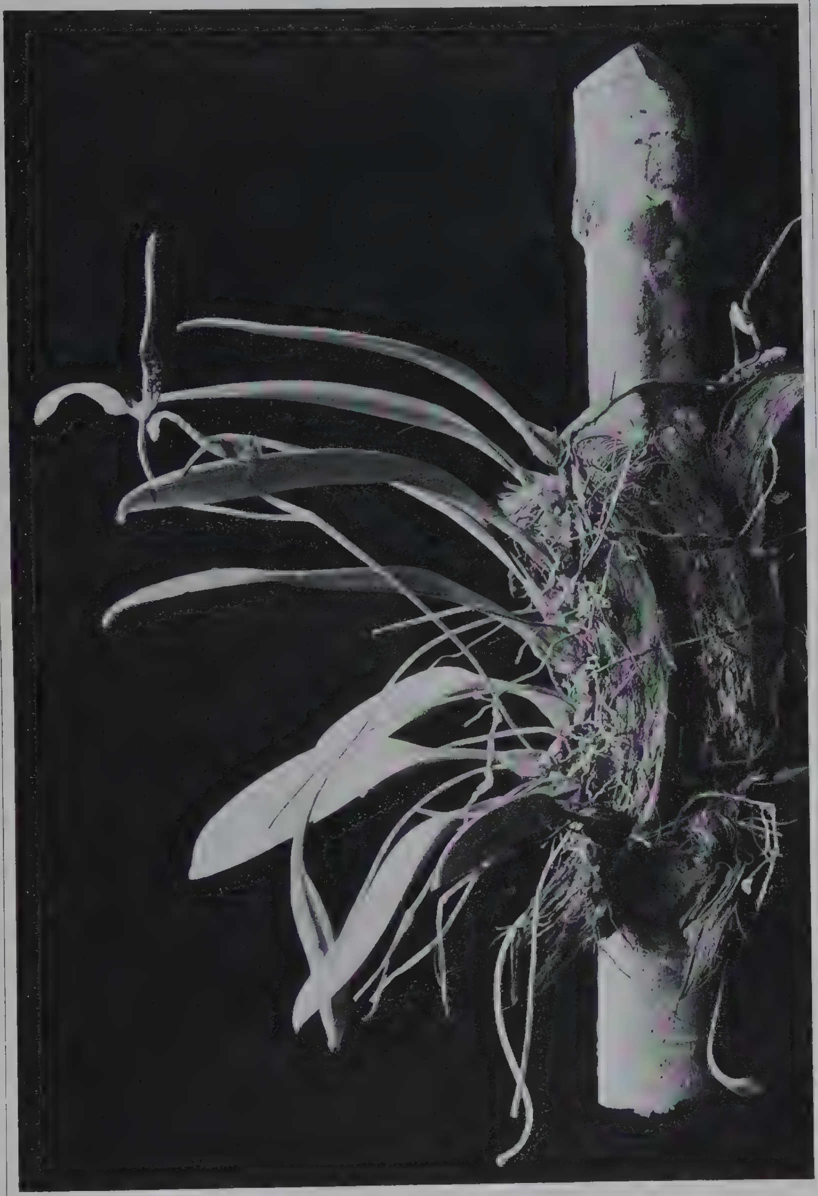

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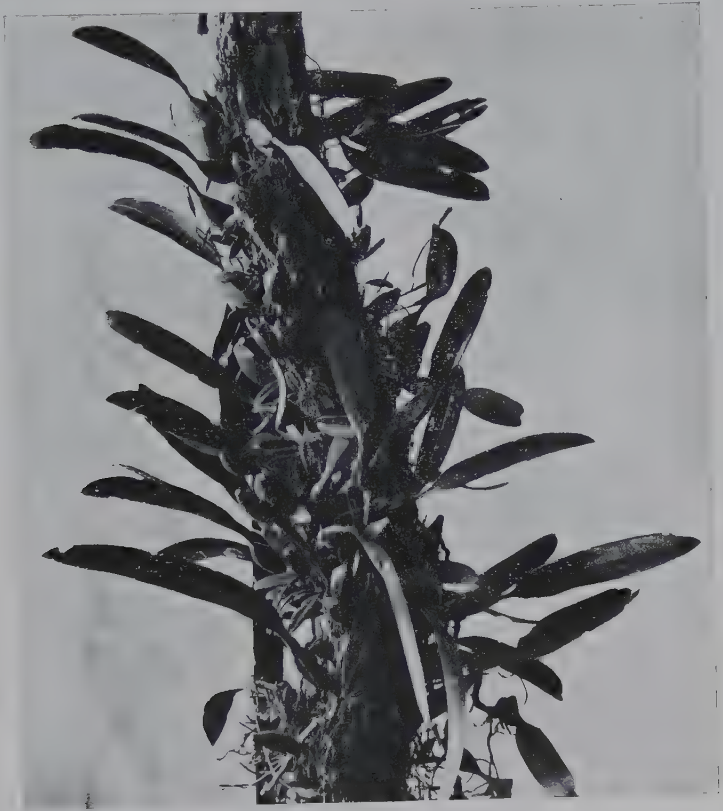

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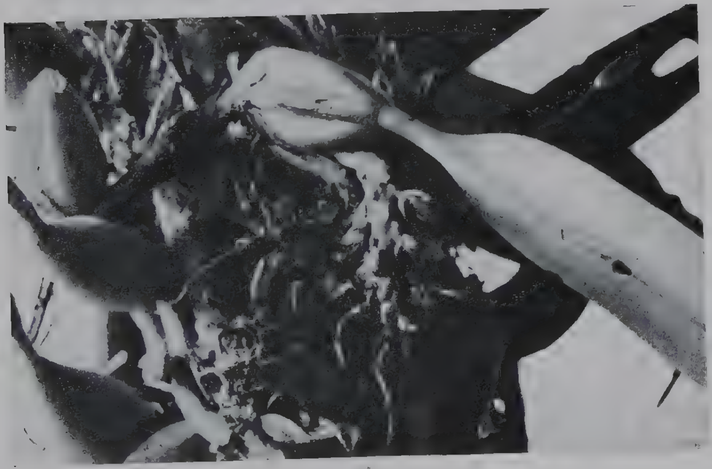

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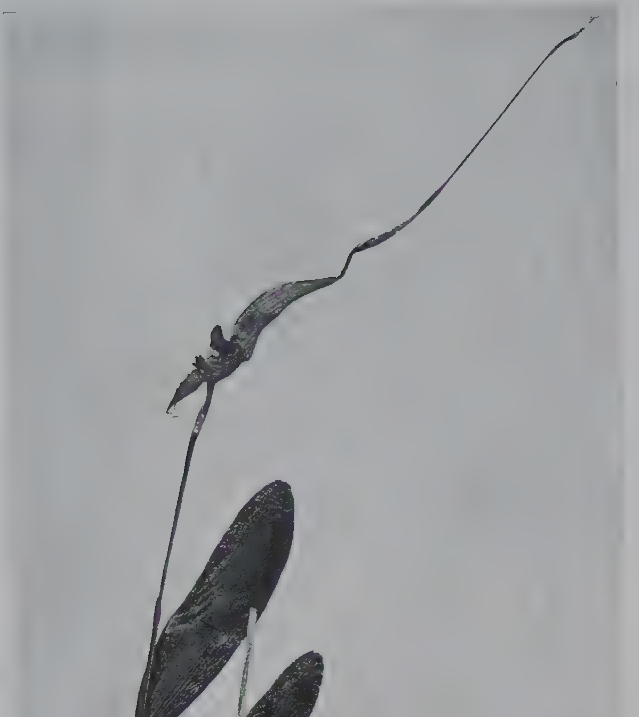

PLATE 16.

ANOMALOUS STEM STRUCTURE IN ARCHANGELISIA FLAVA AND ANAMIRTA COCCULUS FROM THE PHILIPPINES

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EIGHT PLATES AND FOUR TEXT FIGURES

In a previously published work of the writer,(5) the general features of the Menispermaceæ, as reviewed by Solereder, were indicated, but no mention was made by the writer concerning the anomalous structure of the stem observed in some of its representatives. This anomaly occurring in the stems of certain members of this family consists of the appearance of a series of concentric rings or zones of vascular bundles. According to Solereder(7) these successive zones of vascular bundles are secondary and extrafascicular in origin, and De Bary(2) describes them as consisting of wood, bast, and limited cambium which, according to him, may be called rings of growth, and that these rings of growth, following the normal one, arise in the primary outer cortex. This peculiar stem structure is developed by an unusual method of secondary thickening formation and, according to Solereder,(7) has only been observed in the following Menispermaceæ: *Cocculus laurifolius* de Candolle, *C. leaeba* de Candolle, *Chondrodendron platyphyllum* Miers, *C. tomentosum* Ruiz and Pavon, *Pericampylus incanus* Miers, *Anomospermum schomburgkii* Miers, *A. grandifolium* Eichler, *Pachygone domingensis* Eichler, *Abuta amara* Aublet, *A. concolor* Poeppig and Endlicher, *A. imene* Eichler, *A. rufescens* Aublet, and *A. selloana* Eichler. From this list, it is quite clear that no case so far has been worked out extensively among the species under the genera *Archangelisia* and *Anamirta*. Since the stems of *Archangelisia flava* (Linnæus) Merrill and *Anamirta cocculus* (Linnæus) Wight and Arnott from the Philippines exhibit a series of concentric rings of wood more or less similar to those of the species enumerated above, this work was undertaken with the object of revealing further information in connection with the anomalous thickening observed among the stems of some Menispermaceæ.

Archangelisia flava is commonly known as *albustra, abutra,* or *suma.* These names are also applied to *Anamirta cocculus,* although this species is better known as *ligtang.* Both species are large woody vines with stems sometimes reaching 10 centimeters in diameter. They are very similar in their general external features, and in cross sections are both characterized by the presence of a series of concentric rings of wood. The stem of *Archangelisia flava,* however, is commonly distinguished from that of the *Anamirta cocculus* by the yellow color of the wood, whereas that of the latter is white. This yellow color of the stem of *Archangelisia flava* is probably due to the presence of berberine, an alkaloid. According to Wells(8) this species is believed to have a larger percentage of alkaloid than any other plant found in the Philippines. Guerrero(4) states that a decoction of its stem and roots is used by the Filipinos as a febrifuge tonic, emmenagogue or abortive, according to the quantity administered. The stem bark of *Anamirta cocculus* on the other hand, Brown(1) indicates, is made into rope used for tying animals and for hauling. The fruit is employed as fish poison and is also poisonous to other animals.

These two plants are scattered in thickets and forests at low and medium altitudes throughout the Philippines. They are also found in India and Borneo through Malaya to New Guinea.

MATERIAL AND METHODS

The stems of *Archangelisia flava* and *Anamirta cocculus* that furnished the sections for this brief investigation were collected by Mr. Maximo Ramos, of the Bureau of Science, Manila, from the forest of Antipolo, Rizal Province, Luzon, during December, 1928. A young stem from a seedling of *Archangelisia flava* about half a meter high collected by the writer from the foot of Mount Maquiling, Los Baños, Laguna Province, Luzon, in February, 1926, was used in addition to the above material for comparative study.

Several transverse, radial, and tangential sections from 20 to 30 microns thick were prepared, by means of a sliding microtome, from young and mature stems of the two species. These sections were treated with 95 per cent alcohol, stained with safranin, contrasted with Delafield's hæmatoxylin, dehydrated, clarified with xylol, and mounted in balsam. Sections prepared from the stem of a seedling were not stained but simply mounted in glycerin and used for comparison.

For the comparative examination of the individual shape and characteristics of the cells, Schulze's maceration process, as described by Greenish (3) was employed.

ARCHANGELISIA FLAVA (LINNÆUS) MERRILL

Description of the stem.—The young fresh stems of *Archangelisia flava* are from bright green to dark green, smooth, wiry, and occasionally with small axially elongated lenticels. They bear large broadly ovate leaves about 16 centimeters long, with rounded or slightly obtuse base and accuminate apex. Plate 1, figs. 1 and 2, represents the photographs of the fresh and dried leaves, respectively, with small portions of the young stems. When a fresh young stem is cut it exhibits a ring of yellow vascular bundles, and the cut portion exudes a small quantity of yellowish juice, which has a bitterish taste.

The adult stems from 2 to 4 centimeters in diameter have light brown, very thin bark marked longitudinally with shallow furrows and wrinkles. Here and there yellowish white or greenish patches are observed and sometimes small areas of lichens are found on certain portions of the stem. Prominent knots 25 to 40 centimeters apart, which sometimes throw out a branch, also occur. When it is cut transversally, the stem exudes a scanty yellowish sap with a bitterish taste like that of the young stems, and it displays a series of slightly eccentric zones of yellow woody tissue traversed by radiating medullary rays as illustrated on diagrammatic text figure 1 and in the photographs on Plate 1, fig. 1, *a–d*.

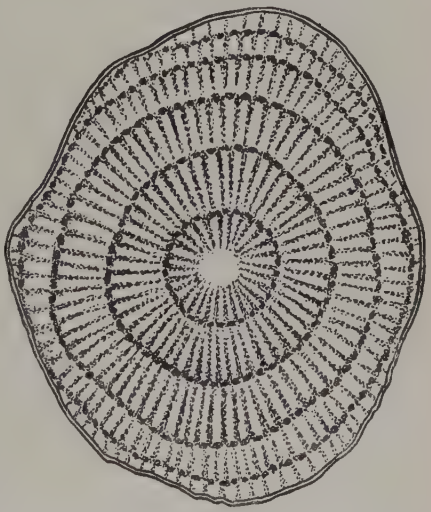

FIG. 1. A diagrammatic view of smoothly cut portion of an adult stem of *Archangelisia flava*, showing the eccentric successive rings of growth. ×1.5.

Structure of the stem.—A thin transverse section of a young stem is circular in outline and is invested by a single layer of colorless rectangular epidermal cells with moderately thickened and cutinized outer cell walls. The cortex is very thin and is composed of two or three layers of thin-walled collenchyma cells and from four to eight layers of small, somewhat tangentially elongated parenchyma cells, both containing a small amount of chloroplastids. The innermost part of the cortex is limited by an inconspicuous starch-sheath. The pericyclic region consists of an undulated continuous sclerenchymatous tissue, which is made up of the arcs of primary hard bast and parenchyma cells, the latter being in the inner part. In the older sections these arcs of primary hard bast are crescent-shaped, but are united by intermediate groups of stone cells into a composite and continuous undulated ring of sclerenchyma, as illustrated in a diagrammatic representation on Plate 2, fig. 3. The primary vascular bundles vary from ten to sixteen, arranged in the form of a ring and separated from one another by broad, primary medullary rays, which are from six to fifteen rows wide and are composed of radially elongated cells. These medullary ray cells have slightly thickened cell walls and are usually loaded with minute, rounded starch grains, while those of the lateral rows are usually filled with prismatic calcium oxalate crystals. The soft bast is not in immediate contact with the inner part of the hard bast; between the two regions there are large and unlignified parenchyma cells, in which no sieve tubes are found. These large parenchyma cells usually contain either a few, slender, prismatic, calcium oxalate crystals or starch grains or a mixture of the two. The sieve tubes are small polygonal cells with very thin walls, and the companion cells are not conspicuous. The primary xylem is separated from the soft bast by a fascicular cambium. It consists chiefly of vessels with large lumina and of wood-prosenchyma with bordered pits. The vessels, which measure from 0.06 to 0.25 millimeter in diameter, possess simple perforations and have bordered pits even where they are in contact with parenchyma, and one or two have spiral or annular thickenings. The pith is wide and circular in outline. It is more or less differentiated by division into two distinct inner and outer regions. The inner region consists of large, polygonal, thin-walled parenchymatous cells with small intercellular spaces. Some of these cells are usually filled with either prismatic raphides, or micro-crystals of calcium oxalate, and starch grains. Occasionally, here and there, a few stone cells are found in the middle region. The outer region, which is limited in the peripheral part by a ring of primary xylem, is built up of several layers of small, polygonal cells with thick walls resembling the transverse section of the long sclerenchymatous cells, but differing from the latter in that the former exhibit small intercellular spaces, and have thinner walls and are not greatly elongated in the longitudinal section, while some of them usually contain calcium oxalate crystals.

Secretory sacs.—Structures that are more or less similar to the bitter-principle sacs observed by the writer(5) in *Tinospora rumphii* Boerlage and *T. reticulata* Miers are found in the cortical region and in the peripheral part of the pith of *Archangelisia flava.* These structures may be called secretory sacs. In cross section they appear very similar to the ordinary parenchyma cells. They are either circular or polygonal in outline with very thin walls and usually contain a yellowish granular substance. In the cortex they are found scattered and intermingled with the cortical parenchyma cells and frequently are found in the outer part of the bast-fiber region or sometimes mixed with the bast fibers as shown on Plate 3, fig. 11. In the peripheral part of the pith the secretory sacs are intermingled with the pith cells with thick walls. They are distinguished, however, from the latter by their thin walls and yellowish granular content. Plate 3, fig. 14, represents a small portion of the transverse section of the pith showing the two kinds of parenchyma cells with thick and thin walls, respectively, and a secretion cell. In the longitudinal section the secretory sacs are axially elongated. They vary in length from 0.45 to 1.2 millimeters and are about 0.03 millimeter in diameter. They are either straight or crooked. Plate 4, figs. 15 and 16, exhibits the characters of the secretory sacs in the longitudinal section.

A critical study of the secretory sacs of *Archangelisia flava* revealed that they originate and develop as a consequence of the gradual and complete disappearance of the cross walls of a row of parenchyma cells in the cortical and pith regions of the stem. They usually occur as independent tubes of various lengths and they possess all the characteristics of the articulated latex tubes, but differ from the latter in that the secretion sacs are not greatly elongated and they neither anastomose nor branch.

The structure of a thin transverse section of an adult stem, however, is much more complicated, on account of its anomalous thickening. The periderm is thin and fissured in various places. It originates from the subepidermal regions. Its formation seems to take place soon after the greatest activity of the fascicular cambium of the primary vascular bundle has started. It begins to develop in circumscribed areas of the stem, and then gradually spreads peripherally, as it penetrates deep into the cortex. The first indication of the formation of the periderm is the slight elongation of the subepidermal cells in a radial direction in a certain part of the stem and this is followed by a tangential division. The inner layer of the resulting daughter cells in this division becomes the meristemic or phellogen region, and the daughter cells towards the epidermis may divide once or twice before they are converted into cork cells. After the phellogenetic centripetal activity in the formation of the periderm has continued for a time, the first phellogen layer thus formed ceases to divide, and becomes replaced by the subsequent layer of cells from the cortical region. This new phellogen layer is in turn replaced by another layer of cells, also from the cortex, after producing in centripetal successions additional increase in the periderm, and so continues successively. While this process is going on, the phellogen layer gradually becomes extended around the stem until its two sides meet forming a complete ring. The periderm cells are tangentially elongated with delicate cell walls, and they alternate with groups of short sclerenchymatous elements or stone cells which contain a brown substance. These stone cells generally form massive irregularly concentric zones, as shown diagrammatically in text fig. 2. A detailed structure of a segment of the periderm is indicated in Plate 2, fig. 4. The outer cortical region is chiefly of parenchymatous character with a few stone cells scattered over it. It is from fifteen to twenty layers of cells deep and is not sharply limited in the inner part by a starch sheath nor by a pericyclic region, as regularly observed in the structure of a transverse section of an ordinary dicotyledonous stem. On the other hand it is bounded on the inner part by one or two layers of stone cells, which form a continuous ring in the outer part of the soft basts of the secondary vascular bundles.

The wood cylinder, or the stele is pluriseriate in character; and it, especially the method of its development, constitutes an essential part of this investigation. Its transverse section exhibits several concentric rings of vascular bundles, which either encircle one another completely on all sides of the transverse section, or develop strongly only on one side. The number of these successive rings varies according to the relative size and age of the stem. Text fig. 2 gives a diagrammatic view of a segment of a transverse section of an adult stem, which shows

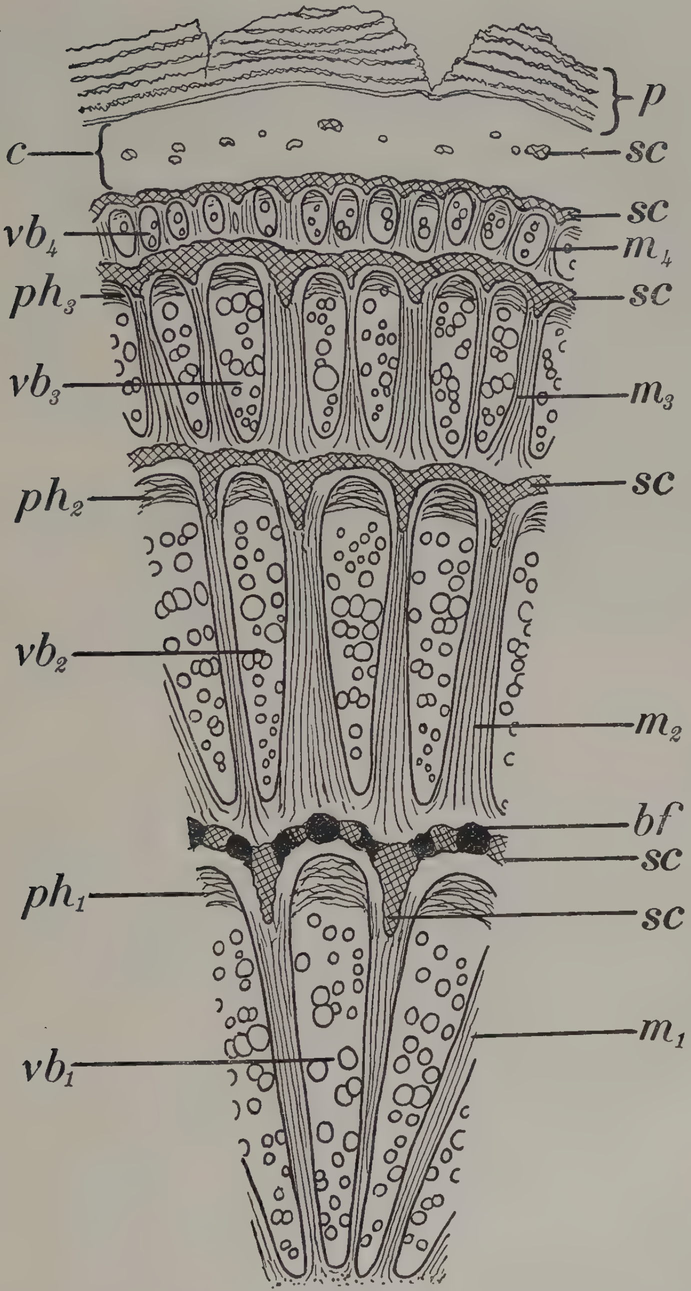
p
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FIG. 2. A diagrammatic camera lucida sketch of a segment of a transverse section of *Archangelisia* adult stem; *p*, periderm; *c*, cortex; *sc*, stone cells; m_1–m_4, medullary rays; vb_1–vb_4, vascular bundles; ph_1–ph_3, phloëm; *bf*, bast fibers.

four successive rings of vascular bundles in various stages of development. These successive rings of growth are separated from one another by groups of stone cells, which are arranged in the form of a continuous and slightly undulated ring. To the naked eye this separation of the individual rings of growth gives the structure the general appearance of a conifer or other dicotyledonous stem that displays characteristic annual rings. A critical examination of the wood of *Archangelisia flava*, however, reveals that its structure is far from being similar to that of a conifer stem or other dicotyledonous stem that exhibits annual rings of growth; for the successive rings of the former consist of groups of vascular bundles, which are arranged in the form of rings and these vascular bundles are separated from one another by broad medullary rays.

The formation of the above-described successive rings of vascular bundles, which constitute the woody part of the stem of *Archangelisia flava*, takes place after a normal secondary thickening of the primary wood and soft bast, in which the activity of the fascicular cambium has already ceased. The growth of the successive rings of vascular bundles is initiated by a group of meristematic cells that develop in the one or two inner layers of parenchyma of the outer primary cortex, which are rich in starch. The first change observed in these parenchyma cells is a radial extension followed by tangential divisions, the latter proceeding usually, but not always, in a centripetal direction. Then a layer of stone cells gradually develops just in the outer part of this meristematic region, which ultimately forms a continuous ring in the peripheral part. Inward from this stone-cell ring the tangential divisions in centrifugal progression of the extra-fascicular meristem continue until the region becomes relatively thick. Plate 2, fig. 5, is a detailed drawing of a segment of the inner cortical region showing the formation of the outer stone-cell ring, and fig. 6 represents an older stage in which the soft bast, *sb*, begins to differentiate from the inner layers. While the tangential divisions coupled with slight radial divisions of the extra-fascicular meristem continue actively, the bast region becomes more pronounced, some of the cells in the inner layers gradually passing over into the thick-walled parenchymatous cells and a secondary cambial layer rises up between the two regions, as illustrated on Plate 2, fig. 7. As the secondary procambial activity is in progress, the cells between the procambial strands become radially elongated and eventually develop into secondary medullary rays, which are usually formed in the same radial direction as the primary ones. By means of the above process a second ring or arc of vascular bundles is formed, which likewise increases in thickness for some time by the activity of its normal cambium. Plate 2, fig. 8, is a young secondary vascular bundle with two vessels, one of which is derived from the activity of its normal cambium and the other by simple differentiation from a parenchymatous cell. The two sides are limited by broad medullary rays, the cells of which contain prismatic calcium oxalate crystals and minute starch grains. After the cambiogenetic increase in thickening of the second ring has continued for some time, the activity of its cambium ceases. Between the outer part of the stone-cell ring of the second layer of vascular bundles and the new stone ring, developed in the inner layers of parenchyma cells of the outer cortex, as was that of the preceding one, another extra-fascicular cambium appears; from which second set of procambial strands there arises a third ring of vascular bundles of the same structure as those of the second short ring of growth. This process is repeated and continues until a big series of successive concentric rings of vascular bundles is formed. Sometimes the extra fascicular meristem, however, does not divide and differentiate at the same time, so that there are unequal divisions or increases in thickness around the peripheral part of the stem. These result in the eccentric position of the pith or eccentric arrangement of the rings. A more or less similar method of anomalous secondary thickening has been observed by the writer(6) in the stem structure of *Chenopodium ambrosioides* Linnæus.

The individual structure of the vascular bundles of the secondary rings is in all respects very similar to that of the primary, but the bast bundles have no sheath of sclerenchymatous cells or bast fibers. The soft bast, or phloëm region, is almost semicircular in transverse section and is composed of sieve tubes, companion cells, and phloëm parenchyma. The phloëm cells towards the periphery are mostly flattened or shriveled, forming an arc-shaped dark zone in which the individual structure of the cells disappears, but those towards the cambial region are more or less polygonal in outline or sometimes nearly rectangular (text fig. 2).

The xylem region consists chiefly of vessels, wood parenchyma, and wood fibers. The vessels are characterized by their large circular or somewhat elliptic lumina and are visible even to the naked eye. Like the vessels observed in the primary vascular bundles, these have bordered pits even in places where they are found in contact with the wood parenchyma cells. Plate 3, fig. 9, is a detailed drawing of a middle portion of the transverse section of a vascular bundle, showing the large vessels surrounded by a mass of wood parenchyma and wood fibers, two of them with young growth of tyloses. The wood parenchyma and wood fibers are polygonal in outline, with thick, slightly lignified, pitted walls. Some of the wood parenchyma, especially of the lateral part of the vascular bundle, contains calcium oxalate crystals. When a piece of the wood is macerated with nitric acid and potassium chlorate, the vessels appear as short cylinders, beaker- or barrel-like in outline, with tangentially elongated simple pits. They measure from 0.05 to 0.3 millimeter in diameter and from 0.2 to 0.4 millimeter in length. The wood fibers or libriform fibers are greatly elongated, with somewhat crooked cells and thick, lignified and pitted walls, both ends of which are pointed. They measure about 0.02 millimeter in diameter and from 0.5 to 1.2 millimeters in length. The bast fibers are very slender, with pointed ends and greatly reduced cavities. They measure about 0.02 millimeter in diameter and from 0.7 to 1.4 millimeters in length. The wood parenchyma, like the wood fibers, have thick and slightly lignified cell walls with simple perforations, but they are shorter and their two ends may be either pointed or blunt. They measure from 0.3 to 0.8 millimeter in length. (Plate 4, fig. 17, *a–g*.) The stone cells are conspicuous and show a diversity of form and size, measuring from 0.03 to 0.1 millimeter in width and from 0.04 to 0.25 millimeter in length.

In the radial sections, through either the vascular bundles or medullary rays, the different zones of wood or short rings of growth are also distinct. This is due to the columns of stone cells that separate one zone from another. These columns of stone cells stand out even to the naked eye. These stone cells are either polygonal in outline or radially elongated with very thick cell walls, which have branched perforations and small lumina.

The secondary medullary rays are similar in structure to the primary medullary rays, although they are usually much wider and are not lignified. The primary ray cells are mostly lignified. In transverse sections they appear uniform in structure, consisting of ten to twenty thin- or thick-walled, pitted, and radially elongated cells, which usually contain calcium oxalate crystals, especially those of the lateral part, and minute rounded starch grains. The parenchyma of the medullary rays, between the masses of bast and the adjoining outermost part of each medullary ray of the xylem, become sclerosed in character. In the radial sections the ray cells appear rectangular in outline, arranged in rows perpendicular to or at right angles to the vascular tissue. The number of the radial rows varies according to the relative size of the medullary ray. In the tangential section on the other hand, the medullary rays appear as boat-shaped areas. The medullary ray cells are polygonal in outline; and those of the lateral part, like those in the transverse section, usually contain large calcium oxalate crystals, which are either prismatic, cubical, rhomboidal, or irregular in shape. The cells in the middle part are filled with minute rounded starch grains or sometimes mixed with a few slender prismatic or rod-shaped calcium oxalate crystals. Plate 3, fig. 12, is a detailed representation of a segment from the lateral part of a transverse section of a medullary ray, showing the different forms of calcium oxalate crystals and starch grains. Plate 3, fig. 13, gives an actual view of a portion of a tangential section of a medullary ray, and displays a large stone cell in the middle part and the characteristic shape of the ray cells with their different forms of calcium-oxalate-crystal content. At the lateral part, the medullary ray is limited by wood parenchymatous cells with bordered pits.

The central pith of an old stem is comparatively the same as that of a young one. It is also circular in outline, measures about 4 millimeters in diameter, and is differentiated into inner and outer zones (Plate 3, fig. 14). Like the central pith of the younger stem, the inner zones are occupied by large polygonal parenchymatous cells with small intercellular spaces, but with thicker, pitted, and slightly lignified cell walls. Some of them contain calcium oxalate crystals in raphides or microcrystals. The only difference is that very often in the pith of the old stem there are some stone cells present. The outer zones, like the outer zones of the younger stem, consist of fourteen to eighteen layers of small polygonal cells with thick, lignified, and distinctly striated cell walls, resembling the transverse sections of the sclerenchymatous cells, but they differ from the latter because they are mostly filled with microcrystals, raphides, or clinorhombic forms of calcium oxalate and because they are not greatly elongated axially.

ANAMIRTA COCCULUS (LINNÆUS) WIGHT AND ARNOTT

The young, fresh stems of *Anamirta cocculus* are almost of the same general appearance as those of *Archangelisia flava*, for they are also from bright green to dark green, smooth, wiry, and with axially elongated lenticels in the older part. The former species differs from the latter, however, in that the leaves are from 12 to 25 centimeters in length, smooth, shiny, and with cordate base and acuminate apex. Plate 5, figs. 18 and 19, gives a view of the external characteristics of the stem and leaves. When a fresh young stem is cut transversely it displays a ring of vascular bundles, which are visible to the naked eye. The cut portion has a whitish color and exudes a scanty whitish or colorless sap with a slightly bitterish taste.

The external characteristics of the adult stems of *Anamirta cocculus* from 2 to 3 centimeters in diameter are also similar to those of *Archangelisia flava*, but the former differ from the latter by their straw to grayish brown color and numerous shallow longitudinal fissures and wrinkles, which sometimes take a spiral direction. *Anamirta cocculus* is not usually covered by greenish patches or by lichens, but prominent knots, which sometimes throw out branches, occur at intervals of about 35 centimeters. The fresh older stems of both *Anamirta cocculus* and *Archangelisia flava*, when cut transversally, display series of concentric rings of wood with conspicuous radiating medullary rays, but the cut portion of the former has whitish wood and exudes a scanty whitish sap, whereas the wood of the latter is yellow and exudes a yellow sap. Plate 5, fig. 18, shows a photograph of four pieces of the older stems of *Anamirta cocculus* of different diameters. When dried the stems shrink slightly, but the fissures and wrinkles become more prominent and the wood turns grayish brown. The wood of *Archangelisia flava*, on the other hand, becomes yellowish brown when dried.

Structure of the stem.—The anatomical structure of a thin transverse section of a young stem of *Anamirta cocculus* is similar to that of the young stem of *Archangelisia flava*. The cortex is also very thin; it is invested in the outer part by a single layer of epidermal cells, and in the inner part by an inconspicuous starch sheath. The pericycle, like that of *Archangelisia*, is composed of a many-layered, undulated, continuous ring of sclerenchymatous cells, formed by arcs of the primary hard and soft bast. The vascular bundles are from twelve to eighteen, collateral, and arranged in the form of a ring with broad medullary rays between them. These primary vascular bundles are very similar in general character to those of *Archangelisia,* but they are relatively longer in a radial direction, are wider, and their microscopical structure is coarser than that of the primary vascular bundles of the latter. Plate 6, fig. 20, gives a diagrammatic view of a young stem of *Anamirta cocculus,* in which the normal secondary thickening in the soft bast and wood and the thickening in the outer cortex have already started. The protoxylem and metaxylem vessels are comparatively larger than the corresponding vessels observed in *Archangelisia flava.* However, they possess the same characteristics in that they have tangentially elongated bordered pits; the medullary rays are from eight to sixteen rows of cells wide; and the medullary ray cells, like those of *Archangelisia flava,* have thick walls, loaded with rhomboidal or prismatic calcium oxalate crystals and minute starch grains.

The central pith of the young stem of *Anamirta cocculus* is also very similar to that of *Archangelisia flava.* The outline is circular, and the pith is differentiated into inner and outer zones. The central pith is comparatively wider than that of *Archangelisia flava,* and the cells composing the inner and outer zones have relatively thinner walls and contain only starch grains, as represented on Plate 7, fig. 32.

Secretory sacs.—According to Solereder,(7) Bailon was the first to observe in *Anamirta* certain structures which he called secretory sacs and that they were described by Blottiere as "Cannaux laticiferes." The works of Bailon and Blottiere are not available. The secretory sacs found in *Anamirta cocculus* are very similar in structure to those observed in *Archangelisia flava.* They originate and develop in the same manner; that is, by the dissolution of the cross walls of certain rows of parenchyma cells. Like the secretory sacs of *Archangelisia* they appear as independent tubes of various lengths, scattered in the cortical region and in the outer zone of the central pith of the stem. The secretory sacs of *Anamirta* are much wider and longer than those of *Archangelisia,* and they contain a granular grayish white substance. They measure about 0.06 millimeter in diameter and from 0.75 to 3 millimeters in length. On Plate 7, fig. 29, is a segment of a radial section through the cortex, showing a portion of a longitudinal view of a secretory sac.

The structure of a thin transverse section of an adult stem of *Anamirta* is similar to that of *Archangelisia.* It is also characterized by a series of concentric rings of vascular bundles of extra-fascicular origin, which are separated from one another by broad medullary rays as indicated in text fig. 3. Text fig. 4 is a diagrammatic view of a segment from a thin transverse section of a stem about 4 centimeters in diameter. The periderm is also very thin and developed from the subepidermal region in the same manner as that of *Archangelisia.* It is fissured in various places and displays the characteristic stone cells forming massive irregularly concentric zones which alternate with the thin-walled and slightly suberized cork cells. Plate 6, fig. 21, gives a detailed view of a segment of a transverse section of the periderm, which is very similar to the corresponding section in *Archangelisia* indicated on Plate 2, fig. 4, but it is coarser in structure than the latter.

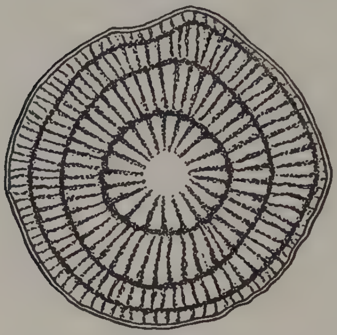
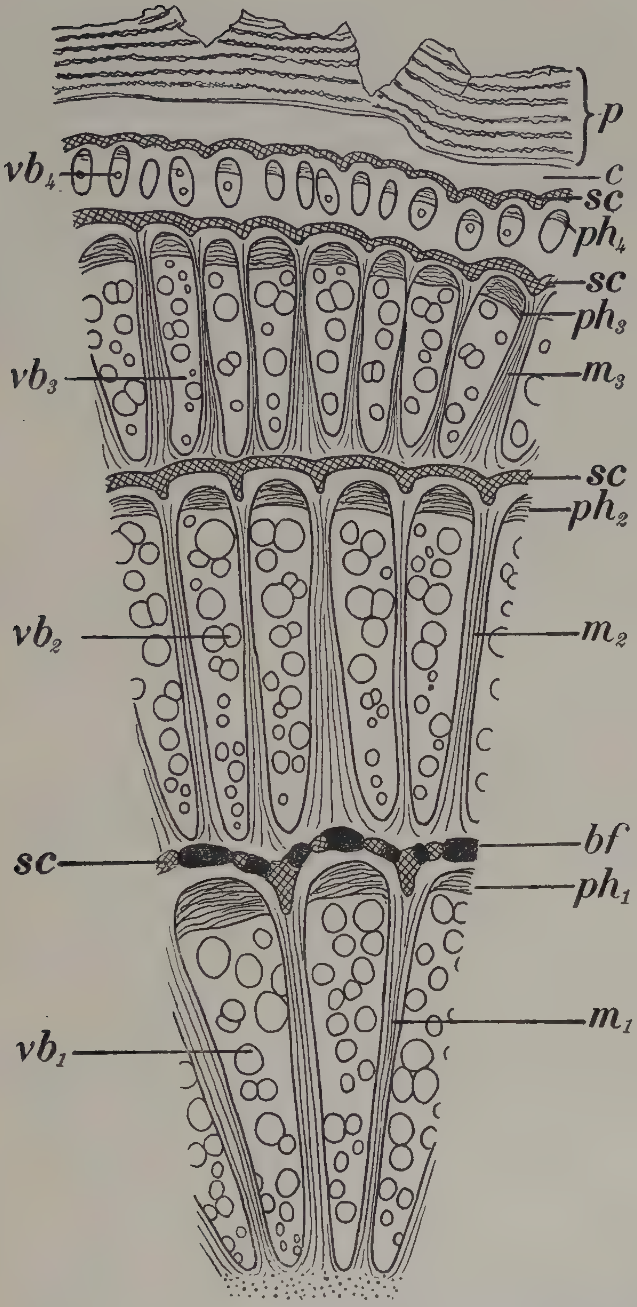


FIG. 3. A diagrammatic representation of the cut surface of the stem of *Anamirta cocculus,* showing the successive rings of growth. × 1.5.

The cortex of an adult stem of *Anamirta* is thinner than that of *Archangelisia.* It consists of ten to fifteen layers of thin-walled parenchymatous cells with a polygonal, rounded, or tangentially elongated outline. Comparatively, there are fewer stone cells in the cortical parenchyma region of *Anamirta* than in that of *Archangelisia.* The secretion cells are not conspicuous in the cortex of an adult stem and presumably they are fewer than in that of younger stems.

The wood cylinder of an adult stem of *Anamirta,* like the wood cylinder of *Archangelisia* of about the same age, is pluriseriate. The first normal ring of thickening surrounds a very wide pith. Its irregular undulating outer margin, similar to that of *Archangelisia,* is marked off from the outer ring of vascular bundles by a composite and continuous sclerenchymatous ring, which is composed of the arcs of the primary hard bast and stone cells. The wood shows practically the same structure as the corresponding region in *Archangelisia* and is arranged in very regular radial rows also, but the vessels are comparatively larger, measuring from 0.06 to 0.35 millimeter in diameter. The broad,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sc
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FIG. 4. A diagrammatic camera lucida sketch of a segment of a transverse section of *Anamirta* adult stem; *p*, periderm; *c*, cortex; *sc*, stone cells; m_1–m_3, medullary rays; vb_1–vb_4, vascular bundles; ph_1–ph_4, phloëm; *bf*, bast fibers.

pluriseriate, primary medullary rays consist of twelve to twenty-four rows of rather thick-walled, pitted, and slightly lignified cells, the lateral rows of which contain calcium oxalate crystals and the middle ones, starch grains. The second ring of thickening which surrounds the first one, is limited in the outer part and separated from the third outer ring by one or two layers of polygonal or radially elongated stone cells. This zone of vascular bundles is derived directly from the inner part of the cortex or from just inside the outer limiting region of the sclerenchymatous ring. The first signs observed in the formation of the secondary ring of thickening in *Anamirta* are the irregular divisions of the three or four inner layers of parenchyma cells of the cortex, which increase the thickness of the cortex. The resulting daughter cells in these divisions are small, with polygonal outline, as indicated on Plate 6, figs. 22, 23, and 24. Following the slight increase in the cortex there appear small groups of cells with thicker walls. These are sometimes arranged in radial rows or in patches; in the places where the corresponding future secondary vascular bundles are to be located as the differentiation and the multiplication of the cells is in progress, secondary procambial strands arise, followed by the development on the outer side of a ring or arc of stone cells. From the procambial strands a second ring, or arc, of vascular bundles is formed and likewise grows in thickness for some time by means of its normal cambium. To this short ring of growth a third and then numerous successive thickening rings are added, always in the same manner. Plate 6, fig. 25, represents a detailed reproduction of a young secondary vascular bundle, showing the structure of the semicircular soft bast region, the early stage of fascicular cambium, and a massive prosenchymatous tissue with a small vessel at one side. In the peripheral and in the inner parts it is limited by layers of stone cells, which are either polygonal or radially elongated, with small cavities and very thick, highly lignified, pitted walls. It is bounded at the two lateral parts by broad medullary rays, which are composed of radially elongated thin-walled cells, some of which contain a few calcium oxalate crystals and some starch grains.

As in *Archangelisia,* the fully developed secondary vascular bundles are similar in structure to the primary vascular bundles. The only difference between these two kinds of bundles is that the soft bast of the former is deprived of sclerenchymatous arcs or hard bast. The phloëm region consists of the same elements; namely, sieve tubes, companion cells, and phloëm parenchyma. The xylem is composed of very large vessels, wood parenchyma, and wood fibers. The vessels like those in the primary xylem possess simple, tangentially elongated perforations. Plate 7, fig. 26, is a segment of a transverse section of a secondary vascular bundle, showing a very large and a small vessel surrounded by a mass of wood parenchyma and wood fibers. The wood fibers of *Anamirta* are distinctly longer and have more-pointed ends than those of *Archangelisia.*

When a portion of the stem of *Anamirta* is treated with nitric acid and potassium chlorate, the most conspicuous elements observed under the microscope are somewhat similar to those of *Archangelisia flava* as shown on Plate 8, fig. 32, *a–f*. The stone cells exhibit a great diversity of form and size. They measure from 0.04 to 0.1 millimeter in diameter and from 0.05 to 0.3 millimeter in length. The wood fibers are slender, measuring about 0.035 millimeter in width and from 0.6 to 1.35 millimeters in length. They appear either straight or crooked with pitted walls. The bast fibers are much longer than those found in *Archangelisia flava,* measure from 1.5 to 2 millimeters in length and about 0.03 millimeter in width, and have a greatly reduced cavity. The wood-parenchyma are characterized by the profuse pittings of their walls. They are from 0.2 to 0.8 millimeter in length and about 0.04 millimeter in width, and are either straight or crooked with slightly tapering ends. The vessels are from 0.05 to 0.5 millimeter in diameter and from 0.15 to 0.55 millimeter in length. They are either beaker-like, barrel-like, or cylindrical with simple tangentially elongated pits, and the medullary ray cells are short with thick pitted walls.

SUMMARY AND CONCLUSIONS

1. The younger stems of *Archangelisia flava* and *Anamirta cocculus* externally are very similar, but internally differ distinctly. The wood of the former is yellow and has a yellowish juice; the wood of the latter is whitish and secretes colorless juice.

2. In the transverse sections both stems exhibit a ring of vascular bundles that are separated one from another by broad medullary rays. The pericycle consists of an undulated continuous sclerenchymatous tissue, which is made up of the arcs of primary hard bast.

3. Secretory sacs, which originate and develop through the disappearance of the cross walls of a row of parenchyma cells, are observed in the cortical region at the outer zones of the central piths of both stems.

4. The secretory sacs of *Archangelisia flava* are smaller and usually more crooked than those of *Anamirta cocculus*.

5. The general characteristics of a thin transverse section of an adult stem of *Archangelisia flava* are very similar to those of the stem of *Anamirta cocculus* of corresponding age. They both exhibit successive concentric or eccentric rings of vascular bundles of secondary and extrafascicular origin, a peculiar and unusual method of secondary thickening formation.

6. The stem of *Archangelisia flava*, besides being yellow, differs as a whole from the structure of the stem of *Anamirta cocculus* in that the tissue of the former in general is finer than that of the latter. The bast fibers, wood fibers, wood parenchyma, and vessels of *Anamirta cocculus* not only possess larger diameters, but also are much longer, comparatively, than those of *Archangelisia flava*.

7. Calcium oxalate crystals of various shapes and sizes are observed in the cortical parenchyma, medullary-ray cells, and pith cells. The calcium oxalate crystals of *Archangelisia*, however, are usually prismatic, clinorhombic, or raphides, whereas those of *Anamirta* are either rhomboidal, cubical, clinorhombic, or raphides.

8. The cells of the peripheral zones of the pith of *Archangelisia* are usually loaded with calcium oxalate crystals in raphides or prismatic forms, with a very few starch grains and only a few secretory sacs intermingled with them, whereas the cells of the corresponding zones in *Anamirta* usually contain starch grains only and relatively more secretory sacs are intermingled with them.

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ILLUSTRATIONS

[Most of the microscopic drawings were prepared by Mr. Mariano Abagon and some are by the author. The photographs are by the Bureau of Science photographer.]

PLATE 1. ARCHANGELISIA FLAVA (LINNÆUS) MERRILL

FIG. 1. Four short fresh stem portions, or pieces, of different diameters showing the successive rings of growth and a small part of a young stem with two fresh leaves.

2. A photograph of a portion of a dried specimen bearing fruit.

PLATE 2. ARCHANGELISIA FLAVA (LINNÆUS) MERRILL

FIG. 3. A diagrammatic view of a transverse section of a young stem; *p*, periderm; *c*, cortex; *sc*, stone cells; *pe*, pericycle; *ph*, phloëm; *x*, xylem; *m*, medullary ray. × 10.5.

4. A small segment of the periderm; *sc*, stone cells; *ph*, phellogen; *c*, cortical parenchyma. × 275.

5. A segment from the inner cortical region, showing the formation of the outer secondary stone-cell ring; *sc*, stone cells; *co*, calcium oxalate crystals. × 165.

6. An older stage corresponding to the region shown in fig. 5, in which the outer secondary stone-cell ring is more or less completed; *sb*, soft bast; *sc*, stone cells. × 165.

7. An early stage of a secondary vascular bundle gradually developed by the differentiation of the parenchyma cells; *ph*, phloëm; *pc*, procambial region; *p*, parenchyma cells with thick walls; *sc*, stone cells; *m*, medullary ray. × 165.

8. A young vascular bundle; *ph*, phloëm; *sc*, stone cells; *fc*, fascicular cambium; *v*, vessel; *m*, medullary ray; *co*, calcium oxalate crystals. × 165.

PLATE 3. ARCHANGELISIA FLAVA (LINNÆUS) MERRILL

FIG. 9. A segment from the middle part of a primary xylem; *v*, vessel; *t*, young stage of tyloses; *wp*, wood parenchyma and wood fibers; *co*, calcium oxalate; *m*, medullary ray. × 165.

10. A portion of the bast-fiber region from the outer part of primary phloëm; *sc*, stone cells; *bf*, bast fibers. × 275.

11. A very small segment taken from the region between the cortex and the bast fibers; *s*, secretion cells; *bf*, bast fibers; *cp*, cortical parenchyma. × 700.

12. A group of medullary-ray cells from a transverse section of the stem; *co*, calcium oxalate crystals; *sg*, starch grains. × 700.

13. A portion of the tangential section of a medullary ray; *m*, medullary-ray cells; *sc*, stone cells; *co*, calcium oxalate crystals; *wp*, wood parenchyma. × 165.

FIG. 14. A portion of the central pith showing the differences between the cells of the inner and outer zones; *s*, secretory sac; *co*, calcium oxalate crystals. × 105.

PLATE 4. ARCHANGELISIA FLAVA (LINNÆUS) MERRILL

FIG. 15. A portion of a radial section through the cortex; *s*, secretory sac; *sc*, stone cells. × 105.

16. Another portion of a radial section through the cortex; *s*, secretory sac. × 105.

17. Different types of cells isolated by Schulze's maceration process; *a*, bast fibers; *b*, wood fibers; *c*, wood parenchyma; *d*, vessels and tracheid, *e*, parenchyma cells with thick walls; *f*, medullary-ray cells; *g*, stone cells. × 105.

PLATE 5. ANAMIRTA COCCULUS (LINNÆUS) WIGHT AND ARNOTT

FIG. 18. Four short fresh stem portions, or pieces, of different diameters and a single fresh leaf beneath.

19. A photograph of a portion of a dried herbarium specimen with fruits.

PLATE 6. ANAMIRTA COCCULUS (LINNÆUS) WIGHT AND ARNOTT

FIG. 20. A diagrammatic representation of a transverse section of a young stem; *p*, periderm; *c*, cortex; *pe*, pericycle; *ph*, phloëm; *x*, xylem; *m*, medullary ray. × 7.

21. A portion of the transverse section of the periderm; *k*, cork cells; *sc*, stone cells; *phe*, phellogen. × 275.

FIGS. 22 to 24. Small segments taken from the inner portion of the cortex, representing the three early stages of the development of the secondary vascular bundles; *pr*, procambial strands; *co*, calcium oxalate crystals; *sc*, stone cells. × 165.

FIG. 25. A young secondary vascular bundle; *sc*, stone cells; *ph*, phloëm; *fc*, fascicular cambium; *wp*, wood parenchyma; *m*, medullary ray; *sg*, starch grains; *co*, calcium oxalate crystals. × 165.

PLATE 7. ANAMIRTA COCCULUS (LINNÆUS) WIGHT AND ARNOTT

FIG. 26. A portion from the middle part of the primary xylem; *v*, vessels; *wp*, wood parenchyma and wood fibers; *m*, medullary ray; *co*, calcium oxalate crystals. × 165.

27. A small portion of the pericyclic region; *s*, secretion sacs; *bf*, bast fibers. × 275.

28. A portion of the pericyclic region after secondary thickening has taken place, showing the bast-fiber region split into small groups of cells; *sc*, stone cells; *bf*, bast fibers. × 61.

29. A lower portion of the tangential section of a medullary ray; *m*, medullary-ray cells; *wp*, wood parenchyma; *co*, calcium oxalate crystals. × 165.

30. Medullary-ray cells from a transverse section of a stem; *co*, calcium oxalate crystals. × 700.

31. A small segment of a radial section through cortical region; *s*, secretory sac; *sc*, stone cells. × 105.

FIG. 32. A small portion of the outer zone of the central pith; *s*, secretory sac; *sg*, starch grains. × 105.

PLATE 8. ANAMIRTA COCCULUS (LINNÆUS) WIGHT AND ARNOTT

FIG. 33. Different forms of cells isolated by Schulze's maceration process; *a*, wood fibers; *b*, bast fibers; *c*, vessels and tracheids; *d*, parenchyma cells with thick and pitted walls; *e*, wood parenchyma; *f*, stone cells magnified. × 105.

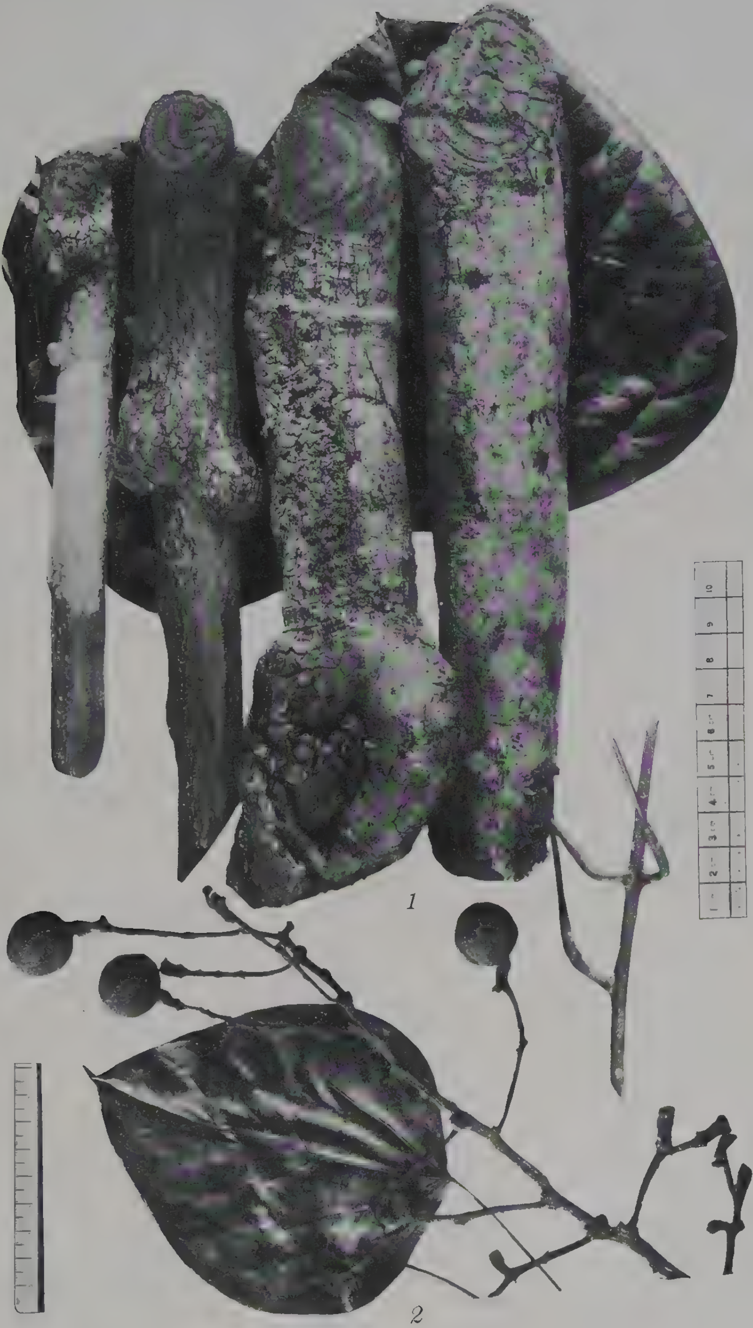
TEXT FIGURES

FIG. 1. A diagrammatic view of smoothly cut portion of an adult stem of *Archangelisia flava*, showing the eccentric sucessive rings of growth. × 1.5.

2. A diagrammatic camera lucida sketch of a segment of a transverse section of *Archangelisia* adult stem; *p*, periderm; *c*, cortex; *sc*, stone cells; m_1–m_4, medullary rays; vb_1–vb_4, vascular bundles; ph_1–ph_3, phloëm; *bf*, bast fibers.

3. A diagrammatic representation of the cut surface of the stem of *Anamirta cocculus*, showing the successive rings of growth. × 1.5.

4. A diagrammatic camera lucida sketch of a segment of a transverse section of *Anamirta* adult stem; *p*, periderm; *c*, cortex; *sc*, stone cells; m_1–m_3, medullary rays; vb_1–vb_4, vascular bundles; ph_1–ph_4, phloëm; *bf*, bast fibers.



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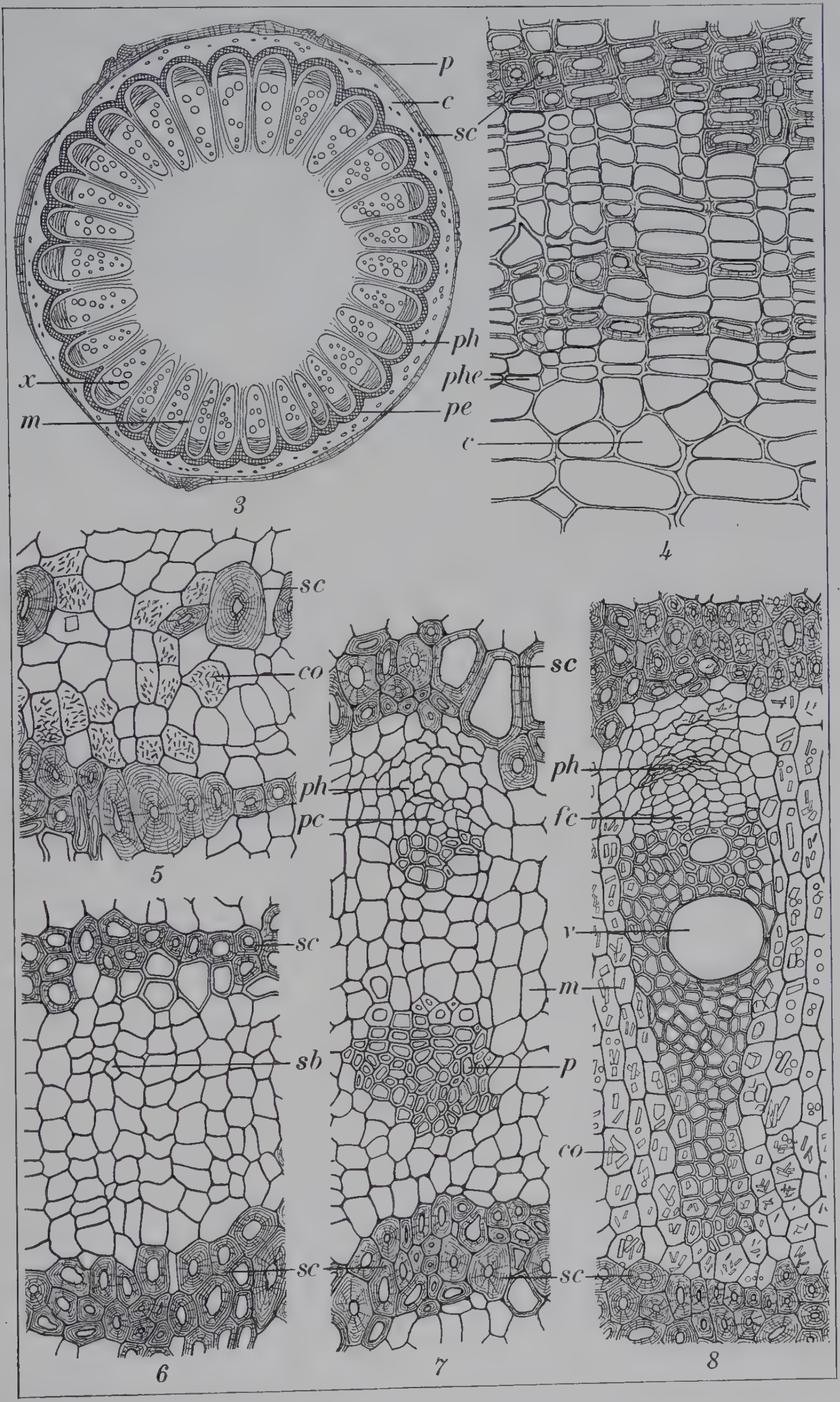
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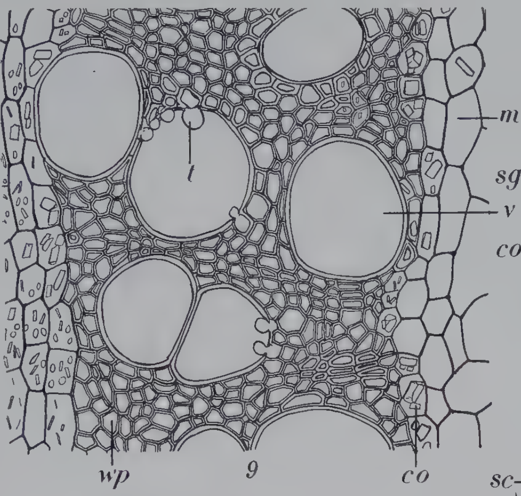
PLATE 2.



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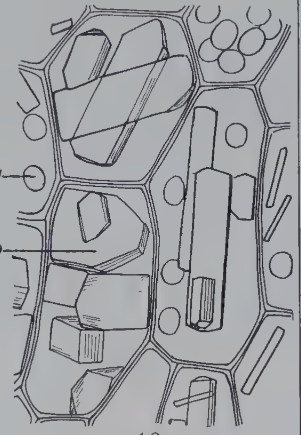

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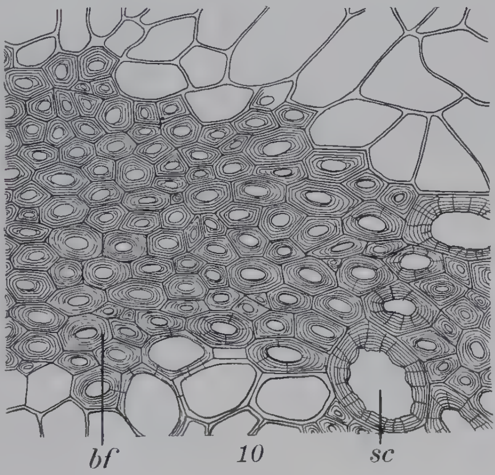

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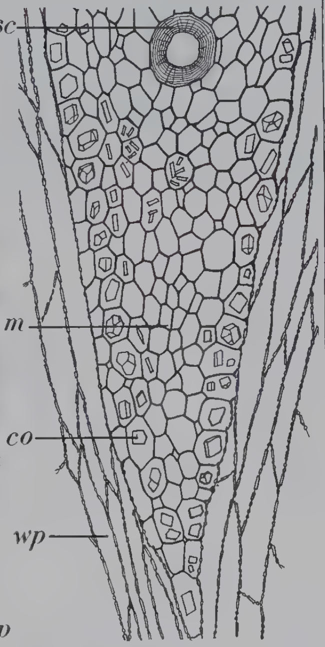

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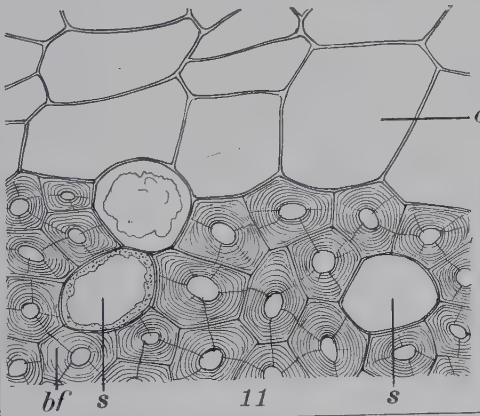

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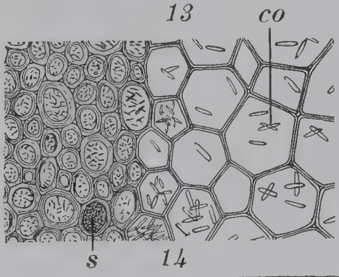

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PLATE 3

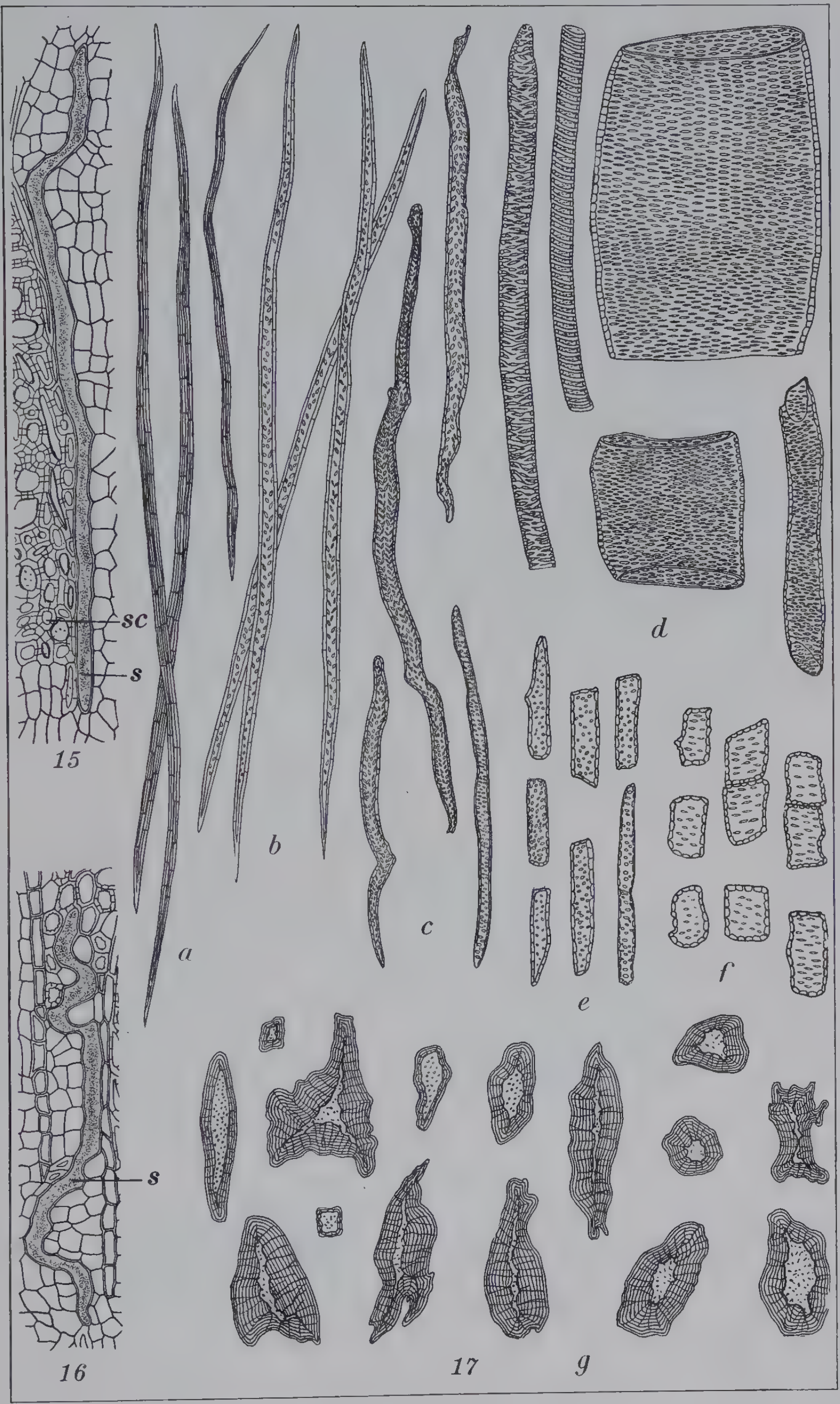
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PLATE 4.

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PLATE 5.

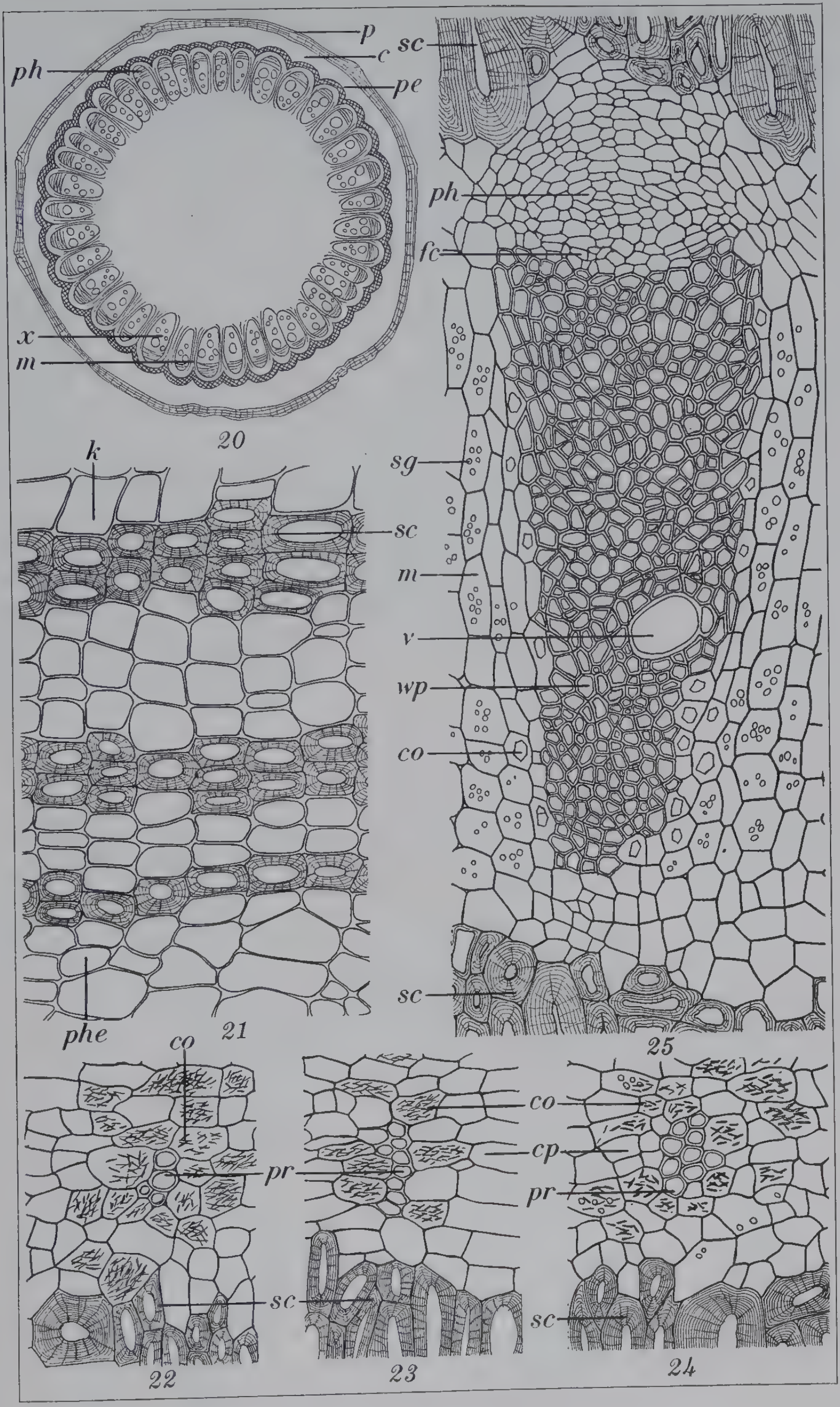
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PLATE 6.

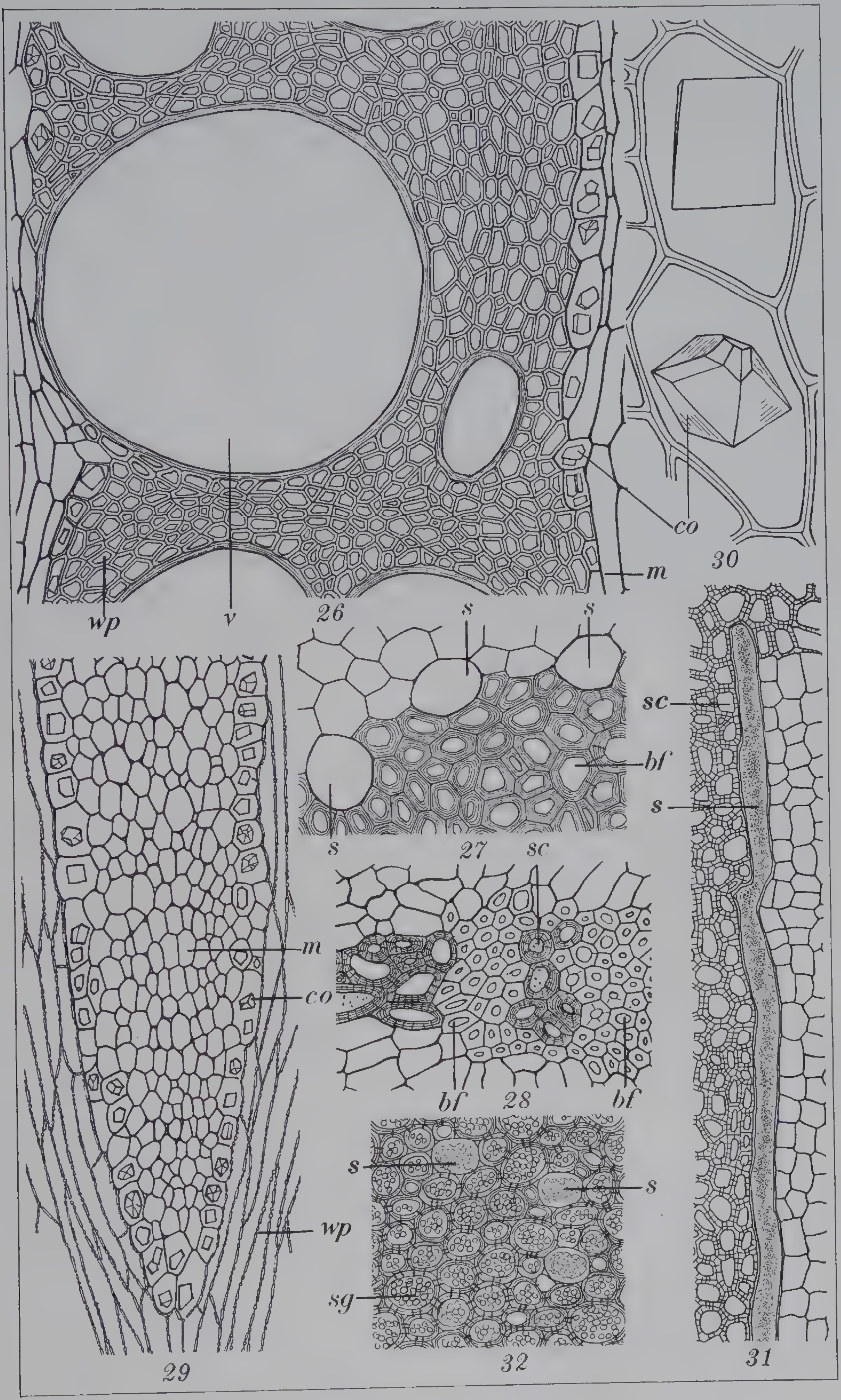
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PLATE 7.

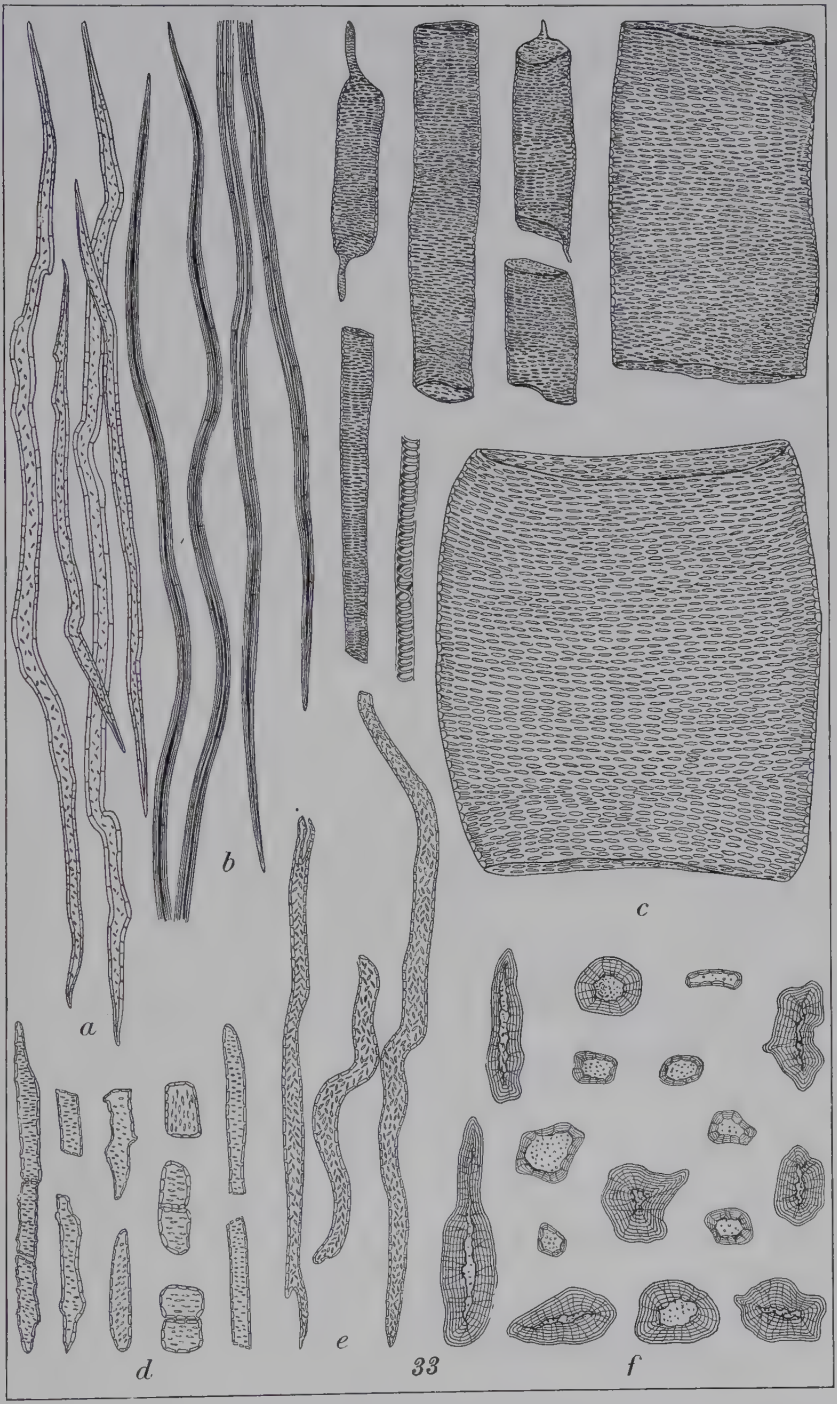

PLATE 8.

THE ISOLATION OF ANONAINE FROM ANONA SQUAMOSA LINNÆUS

By FELICIANA R. REYES and ALFREDO C. SANTOS
Of the Department of Pharmaceutical Chemistry
University of the Philippines, Manila

Anona squamosa Linnæus, commonly known as "ates," is a plant cultivated throughout the Philippines. According to Tavera[1] the powdered seeds are used to kill parasites of the human body, especially lice.

Very little is known of the chemistry of the plant. N. Trimourti[2] obtained from the leaves a white powdery base in the amount of 0.4 per cent calculated as chloroplatinate. It appears that Trimourti did not obtain the pure base.

In a recent communication, one of us[3] reported the isolation of anonaine from the bark of *Anona reticulata* Linnæus, a base melting at 122 to 123°. Based on the result of the elementary analysis, molecular-weight determinations, and the analysis of the hydrochloride and the chloroplatinate the formula $C_{17}H_{16}NO_3$ has been adopted for anonaine.

To determine the possible relation between the base of Trimourti and anonaine, the bark of *Anona squamosa* has been chemically examined and found to contain a crystalline alkaloid melting at 122 to 123°. In this communication it is further shown that this base is identical with anonaine. The melting points of the free base from *Anona squamosa* and that of anonaine as well as their hydrochlorides and chloroplatinates agree with each other. No depression was observed when the melting points of the mixed free bases, and other salts mentioned, were determined as shown in the following table:

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[1] Tavera, T. H. Pardo de, Plantas medicinales de Filipinas. Madrid (1892) 21.

[2] Journ. Ind. Inst. 7 (1924) 232–34.

[3] Philip. Journ. Sci. 43 (1930) 561.

	Anonaine from *Anona reticulata.*	Base from *Anona squamosa.*	Melting point of mixture.
	°*C.*	°*C.*	°*C.*
Base	122–23	122–23	122–23
Hydrochloride (decomposed)	287–88	287–88	287
Chloroplatinate (decomposed)	248.5	247	248.5

It would now be very interesting to examine *Anona muricata* Linnæus to find out the relation to anoniane of the amorphous alkaloid mentioned by Callan and Tutin.[4]

EXPERIMENTAL

For the isolation of the alkaloid the method[3] used in the isolation of anonaine from *Anona reticulata* Linnæus has been followed. Alkaloid, weighing 0.258 gram; was obtained from 1.8 kilograms of material; yield, 0.0143 per cent.

After several recrystallizations of the hydrochloride the free base was prepared and found to melt at 122 to 123°. The mixed melting point with authentic anonaine was 122 to 123°.

In the previous communication the melting points of anonaine hydrochloride and anonaine chloroplatinate as obtained from *Anona reticulata* Linnæus were not reported. These constants have been determined and are 287 to 288° for anonaine hydrochloride and 248.5° for anonaine chloroplatinate. The melting points of the hydrochloride and of the chloroplatinate of the base under examination were 287 to 288° and 247°, respectively. Mixed melting point with authentic anonaine hydrochloride and anonaine chloroplatinate gave 287° and 248.5°, respectively.

MANILA, *July 19, 1930.*

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[3] Philip. Journ. Sci. 43 (1930) 561.

[4] Pharm. Journ. § IV 33 (1911) 743.

AN AUTOMATIC VACUUM PRESSURE REGULATOR[1]

By Howard Irving Cole

Chief Chemist, Culion Leper Colony, Philippine Health Service

ONE TEXT FIGURE

In the course of separating the ethyl esters of hydnocarpic and chaulmoogric acids by fractional distillation, great difficulty was experienced in maintaining a constant pressure (of 20 millimeters) by means of a mercury trap. An automatic vacuum regulator was constructed to be used on a 110-volt direct current. It has now been in constant operation for two years with very satisfactory results. It will maintain a vacuum within ± 0.5 millimeter. In principle it is similar to the regulator of Miller and McKinney[2] but contains several modifications. Text fig. 1 shows a sketch of the apparatus. The pressure is determined by the difference in height of the mercury columns in tubes *a* and *b* which are in the same mercury well, *b* being a barometer.[3] Tube *a* has the same internal diameter as *b* but is constricted to 0.5 millimeter at the bottom to prevent bouncing of the column of mercury. The pressure is regulated by moving either the tube *a* or the wire *c* up or down. To prevent excessive sparking a 1-microfarad condenser is placed across the circuit between *c* and *d*. To reduce the current, a 20-watt lamp, *f*, is placed in series with the electromagnet *g*; *h* is the base of a No. 22 hypodermic needle; *i* is one-half of a No. 0 rubber stopper; *m* is a screw cock; *j* and *k* are adjustable; *l* is a piece of clock spring rivetted to *n* and screwed to a block.

OPERATION

When the vacuum becomes high enough to make contact at *c* it closes the circuit; *n* is drawn to the magnet, thus opening *h* and allowing air to enter the system. The mercury falls in *a*,

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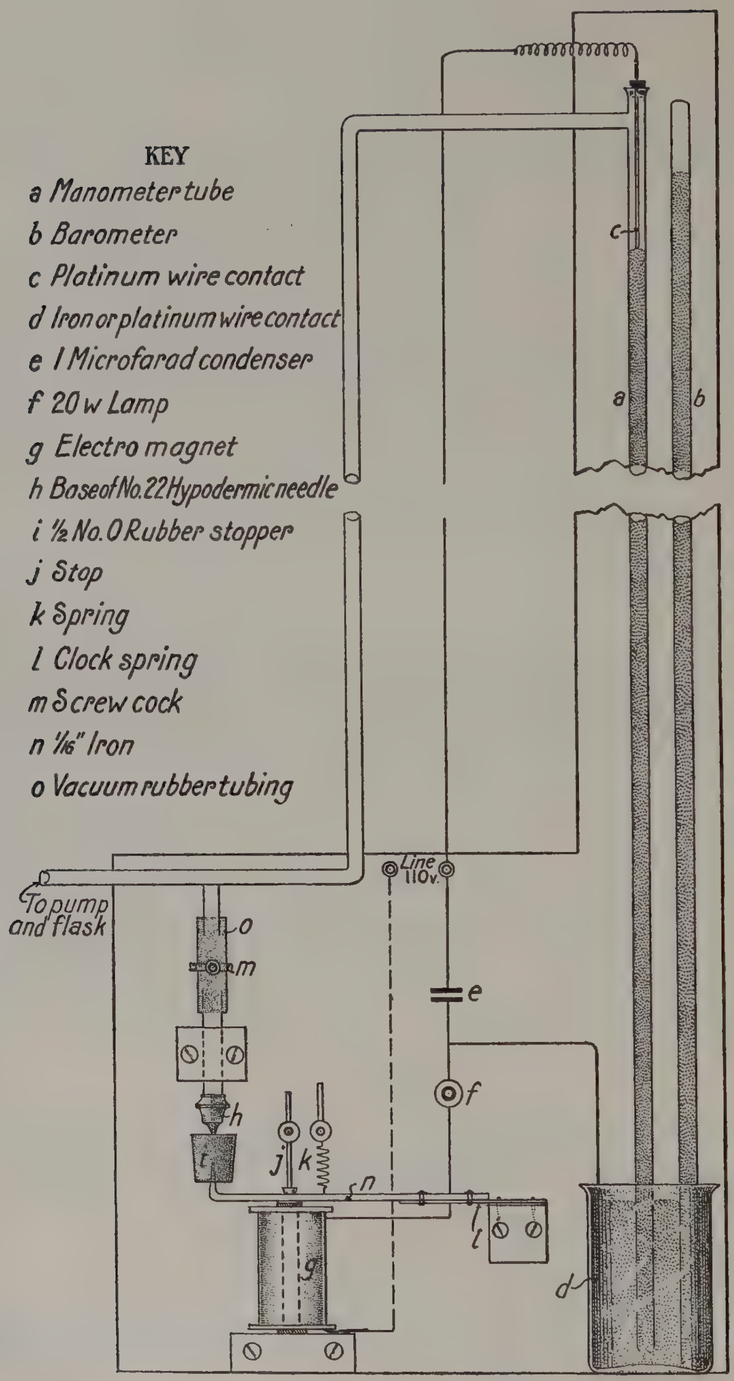
[1] Published with the approval of the Director of Health.

[2] Journ. Ind. Eng. Chem. 20 (1928) 552.

[3] By using this type of manometer corrections for temperature and capillarity can be ignored. The vacuum can be immediately adjusted to change of barometric pressure by simply maintaining the desired difference in the two columns of mercury.


KEY
a Manometer tube
b Barometer
c Platinum wire contact
d Iron or platinum wire contact
e 1 Microfarad condenser
f 20 w Lamp
g Electro magnet
h Base of No. 22 Hypodermic needle
i ½ No. 0 Rubber stopper
j Stop
k Spring
l Clock spring
m Screw cock
n 1/16" Iron
o Vacuum rubber tubing
c
a
b
Line 110v.
To pump and flask
o
m
e
f
h
i
j
k
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d

FIG. 1. Vacuum pressure regulator.

breaking the circuit, thus closing *h*. If the opening in *h* is too large, an excessive amount of air is introduced letting the vacuum fall too much. Various-sized openings were tried, but it was found that a method of regulating the amount of air introduced was necessary in any case. This is accomplished by the screw cock *m* acting on a piece of heavy vacuum rubber tubing *o*. By regulating *m* the variation in vacuum can be controlled to within less than 0.5 millimeter.

With slight modifications this apparatus can be run on a battery instead of a 110-volt direct current.

ILLUSTRATION

TEXT FIG. 1. Vacuum pressure regulator. 415

TREMATODE PARASITES OF PHILIPPINE VERTEBRATES, III: FLUKES FROM FISH AND REPTILES

By Marcos A. Tubangui

Of the Division of Biology and Serum Laboratory
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ONE PLATE

FISH TREMATODE

ORIENTOCREADIUM BATRACHOIDES g. et sp. nov. Plate 1, fig. 1.

This trematode is a common parasite of the fresh-water fish *Clarias batrachus* (Hamilton-Buchanan). It is referred to the family Allocreadiidæ Stossich, 1904, emend. Winfield, 1929, because of the following characteristics: The excretory bladder is single and bag-shaped; the genital pore is directly preacetabular; the ovary is pretesticular, behind but not far from the acetabulum; the testes are proximate in the posterior half of the body; and the uterus is moderately long, extending to the posterior end of the body. As far as can be ascertained from the available literature, it cannot be assigned to any of the existing genera included in that family; hence, the new genus *Orientocreadium* is hereby proposed to receive it. It bears a certain resemblance to *Plesiocreadium typicum* Winfield, 1929, but it differs from the latter in the position of its seminal vesicle outside of the cirrus sac, the presence of a prepharynx, the shortness of the œsophagus, and in the posterior extent of the vitelline glands.

Generic diagnosis.—Allocreadiidæ: Small distomes with moderately developed suckers; oral sucker simple, subterminal; acetabulum at one-third of body length from anterior end. Cuticle provided with spines. Pharynx separated from mouth by short prepharynx; œsophagus short; intestinal cæca long, reaching to near posterior end of body. Testes median, tandem, postovarial, postequatorial; cirrus sac beside acetabulum, large, muscular, inclosing only well-developed pars prostatica and cirrus; seminal vesicle large, outside of cirrus sac; genital pore median, directly preacetabular. Ovary median or very close to median line, between acetabulum and first testis; uterus moderately long, postequatorial, reaching to near posterior end of body; vitellaria moderately developed, extending to posterior end of body; eggs small, fairly numerous.

Type species, *Orientocreadium batrachoides* sp. nov.

Description of type species.—Body small, elongate, rounded at both ends, measuring 1.74 to 2.12 by 0.41 to 0.54 millimeters; maximum width behind equator of body, usually across testes. Cuticle armed with small spines, which become sparse posteriorly and may be absent altogether at level behind first testis. Both suckers fairly well developed and nearly equal in size; oral sucker subterminal, 0.17 to 0.19 millimeter in diameter; acetabulum 0.16 to 0.20 millimeter in diameter, at one-third of body length from anterior end, or very close to that level. Mouth subterminal, separated from pharynx by short prepharynx; pharynx 0.08 to 0.13 by 0.11 to 0.13 millimeter in size; œsophagus very short, 0.03 to 0.07 millimeter long; intestinal cæca moderately wide in diameter, reaching to posterior end of body.

Testes unequal, median, tandem, postovarial, postequatorial; first testis globular, smaller than second testis, 0.15 to 0.20 by 0.13 to 0.17 millimeter in size; second testis ovoid, 0.16 to 0.26 by 0.11 to 0.17 millimeter in size; cirrus sac large, muscular, to one side of acetabulum, 0.30 to 0.33 by 0.07 to 0.09 millimeter in size, inclosing well-developed pars prostatica and long cirrus; seminal vesicle large, 0.24 to 0.26 by 0.07 to 0.08 millimeter in size, located outside of cirrus sac. Common genital opening directly preacetabular, at or slightly to one side of median line.

Ovary oval, median, at equator of body, behind but not far from ventral sucker, 0.16 to 0.19 by 0.12 to 0.13 millimeter in size; shell gland small, to one side of median line behind ovary; Laurer's canal present; receptaculum seminis absent, proximal portion of uterus being dilated and serving as seminal receptacle; uterus moderately developed, postequatorial, extending posteriorly to near caudal end of body and laterally outside of cæca; vitellaria in follicles being often united so as to form a sort of lattice work and extending from anterior level of ovary to posterior end of body where follicles from two sides unite. Eggs fairly numerous, small, operculated, amber-yellow, 24.7 to 31.5 by 18.0 to 20.2 microns in size.

Excretory bladder simple, bag-shaped, opening outside through excretory pore at posterior tip of body.

Specific diagnosis.—Orientocreadium: Small, elongate, rounded at both ends, 1.74 to 2.12 by 0.41 to 0.54 millimeters in size; greatest diameter behind middle of body length, usually across testes; prepharynx and œsophagus both very short; testes with smooth margins, unequal in shape and size, anterior one being globular and posterior oval; seminal vesicle large, outside of cirrus sac; ovary oval; vitellaria in connected follicles extending from anterior level of ovary to posterior end of body; eggs operculated, amber-yellow, 24.7 to 31.5 by 18.0 to 20.2 microns in size.

Host.—Clarias batrachus (Ham.-Buch.).

Location.—Intestine.

Locality.—Laguna de Bay, Luzon.

Type specimens.—Philippine Bureau of Science parasitological collections, No. 36.

REPTILIAN TREMATODES

MESOCOELIUM MEGGITTI Bhalerao, 1927. Plate 1, fig. 3.

Numerous specimens of this fluke were collected on two occasions from the small intestine of the common ground lizard *Mabuia multifasciata* Kühl. Except for minor details, they resemble the form described by Bhalerao (1927) from a Rangoon lizard, *Mabuia dissimilis,* under the name *Mesocoelium meggitti.* The differences, it is believed, are due to the age of the specimens, those of Bhalerao being apparently young mature specimens. Our Philippine material is a mixture of young and fully developed mature specimens, the former tallying with Bhalerao's description.

Description.—Body elongate, flat, broader anteriorly than posteriorly, measuring 1.02 to 3.30 by 0.36 to 1.03 millimeters. Cuticle beset with minute spines at anterior half or two-thirds of body length. Oral sucker ventroterminal, 0.14 to 0.27 millimeter in transverse diameter; acetabulum between anterior and middle thirds of body length, much smaller than oral sucker, 0.07 to 0.17 millimeter across. Mouth ventroterminal, separated from pharynx by very short prepharynx; pharynx 0.05 to 0.10 by 0.06 to 0.13 millimeter in size; œsophagus 0.02 to 0.08 millimeter long; intestinal cæca simple, terminating at level between middle and last thirds of body length or very close to that level.

Reproductive glands grouped around acetabulum. Testes oval, slightly preovarial on each side of acetabulum, the one on the same side as the ovary usually more advanced anteriorly than the other; they are nearly equal in size, measuring 0.08

to 0.17 by 0.07 to 0.12 millimeter; cirrus sac small, median, between pharynx and acetabulum, 0.25 to 0.27 by 0.04 to 0.05 millimeter in size, inclosing small seminal vesicle, pars prostatica, and cirrus. Common genital opening median or slightly to one side of median line, at middle level of œsophagus.

Ovary oval, to one side of median line, post-testicular, though often occurring almost at the same level as opposite testis, measuring 0.08 to 0.20 by 0.07 to 0.15 millimeter; shell gland appears diffuse, close to mesial side of ovary; receptaculum seminis and Laurer's canal present. Uterus mostly postovarial, reaching to near posterior end of body; occurs in two loops, each with numerous transverse loops:—a descending loop filled with colorless unripe eggs and an ascending loop filled with yellowish to dark brown mature or ripe eggs. Vitellaria in distinct follicles, extending on both sides of body from posterior level of oral sucker to near one-third the distance of body length from posterior end. Mature eggs operculated, 30 to 36 by 20 to 24 microns in size.

Excretory pore at posterior end of body; excretory bladder a narrow or dilated canal extending from excretory pore at posterior end of body to behind shell gland, where it divides into two lateral branches.

Host.—*Mabuia multifasciata* Kühl.

Location.—Intestine.

Locality.—Los Baños, Laguna Province, Luzon.

EUPARADISTOMUM VARANI g. et sp. nov. Plate 1, fig. 2.

Seven mature specimens of this parasite were collected from the gall bladder of a young chicken-eating lizard, *Varanus salvator* Linn., which was caught near Molawin Creek, Los Baños, Luzon, November 17, 1929. In the fresh state, they closely resemble members of the genus *Paradistomum,* but a study of the internal anatomy after the flukes had been stained and mounted, revealed the following differences, which appear sufficiently important to place the species in a new genus in the trematode family Dicrocœliidæ Odhner, 1911: (*a*) The testes, instead of being behind the acetabulum, as is usually the case among the members of the family Dicrocœliidæ, are located anterior of that organ; (*b*) the uterine coils are very profuse, being distributed throughout most of the body substance; (*c*) the acetabulum is equatorial in position; and (*d*) the cirrus sac is far behind the pharynx, being located at a level about equidistant between that organ and the ventral sucker.

Generic diagnosis.—Dicrocœliidæ: Body relatively broad and flat, attenuated at both ends. Cuticle smooth. Acetabulum at or very close to equator of body, weaker than oral sucker. Genital pore median, far in front of acetabulum. Testes symmetrical, directly preacetabular; cirrus sac small, about midway between two suckers. Ovary close to median line, directly postacetabular; receptaculum seminis small; uterine coils profusely distributed throughout most of body substance, not clearly arranged into ascending and descending loops; vitellaria moderately developed, extracæcal. Excretory bladder Y-shaped.

Type species, *Euparadistomum varani* sp. nov.

Description of type species.—Body relatively broad, flat, more pointed posteriorly than anteriorly, 2.0 to 2.9 by 1.3 to 1.7 millimeters in size. Cuticle smooth. Oral sucker subterminal, 0.69 to 0.79 millimeter in transverse diameter; acetabulum at or very close to equator of body, smaller than oral sucker, 0.35 to 0.45 millimeter across. Mouth subterminal; prepharynx absent; pharynx 0.19 to 0.21 by 0.22 to 0.24 millimeter in size; œsophagus 0.15 to 0.20 millimeter long, bifurcating at level about midway between genital pore and pharynx; intestinal cæca much dilated, not quite reaching to caudal end of body.

Testes small, oval, with smooth margins, symmetrical, intercæcal, directly preacetabular, measuring 0.24 to 0.28 by 0.14 to 0.22 millimeter; cirrus sac small, elongated, 0.23 by 0.07 millimeter in size, in median line about equidistant between oral and ventral suckers, inclosing slightly coiled seminal vesicle, pars prostatica, and protrusible cirrus. Common genital opening behind œsophageal bifurcation.

Ovary oval, with smooth borders, slightly larger than testes, 0.24 to 0.31 by 0.15 to 0.20 millimeter in size, directly postacetabular, close to median line; shell gland distinct, median, near posteromesial border of ovary; receptaculum seminis small, Laurer's canal present; uterine coils very profusely distributed throughout most of body substance, not clearly arranged into ascending and descending loops; vitellaria moderately developed, in lateral margins of body, extracæcal, extending from posterior level of cirrus sac to termination of intestinal cæca or slightly beyond that level. Mature eggs amber yellow, operculated, with a sort of neck below the operculum, measuring 39.5 to 45.0 by 22.5 to 24.7 microns.

Excretory bladder Y-shaped; excretory pore at posterior tip of body.

Specific diagnosis.—Euparadistomum: Body broad, flat, attenuated at both ends, but more pointed posteriorly, 2.0 to 2.9 by 1.3 to 1.7 millimeters in size; cuticle smooth; acetabulum at equator of body; cirrus sac small, in median line about midway between suckers; testes oval, symmetrical, directly preacetabular; ovary directly postacetabular, close to median line; uterus very well developed and distributed throughout most of body substance; vitellaria extracæcal, extending from posterior level of cirrus sac to termination of intestinal cæca or slightly beyond that level; eggs numerous, operculated, with distinct necks, 39.5 to 45.0 by 22.5 to 24.7 microns in size.

Host.—Varanus salvator Linn.

Location.—Gall bladder.

Locality.—Los Baños, Laguna Province, Luzon.

Type specimens.—Philippine Bureau of Science parasitological collections, No. 37.

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WINFIELD, G. F. Plesiocreadium typicum, a new trematode from Amia calva. Journ. Parasit. **16** (1929) 81–87.

ILLUSTRATIONS

[The lettering of the figures in this and in future papers on Philippine trematodes will be similar to that used in Trematode parasites of Philippine vertebrates, II, See Philippine Journal of Science 44 (1931).]

PLATE 1

FIG. 1. *Orientocreadium batrachoides* g. et sp. nov., ventral view. (Drawing by M. Ligaya.)

2. *Euparadistomum varani* g. et sp. nov., ventral view.
3. *Mesocoelium meggitti* Bhalerao, 1927, ventral view.

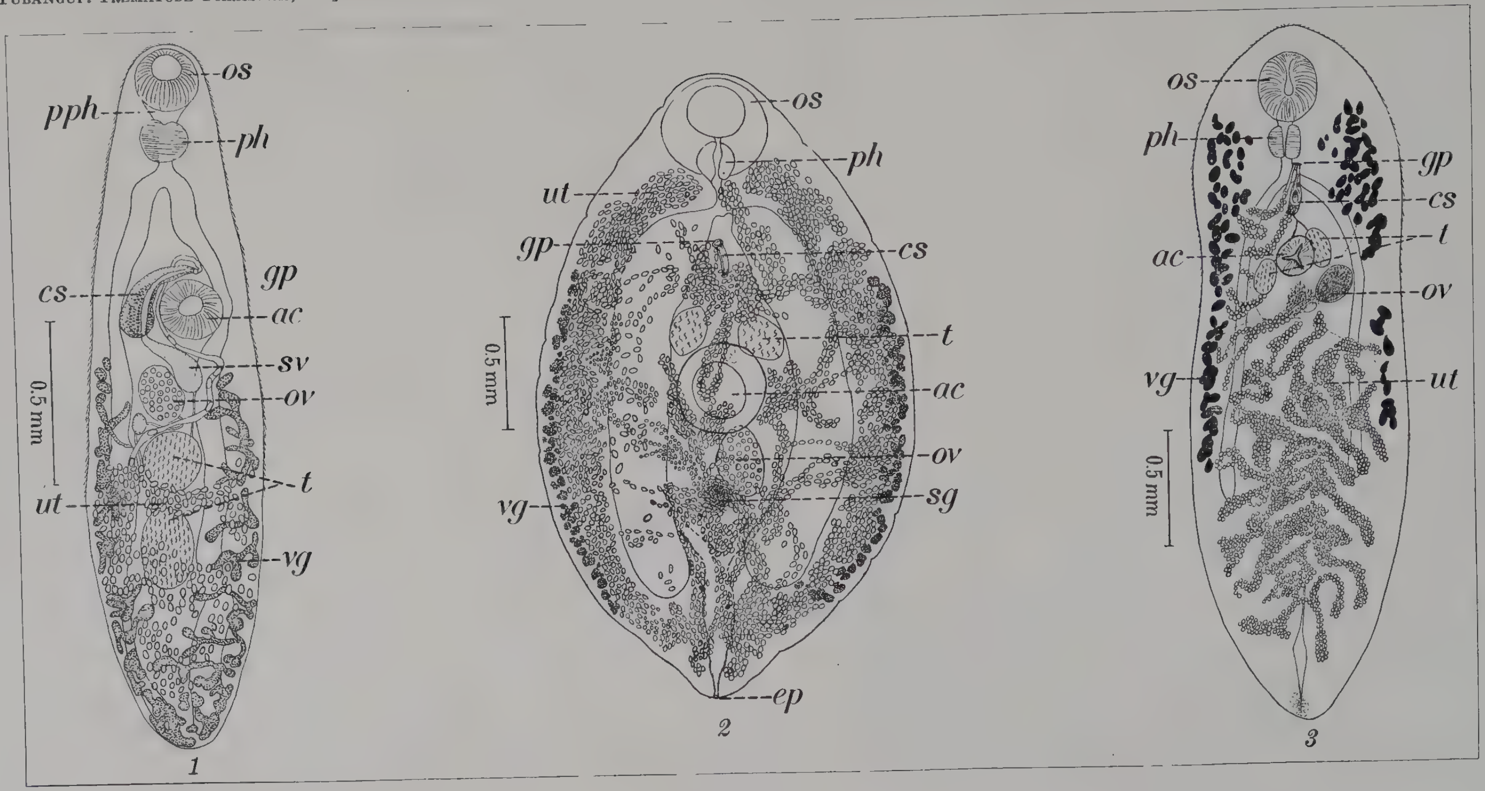
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PLATE 1.

THE BARBIROSTRIS-HYRCANUS GROUP OF THE PHILIPPINE ANOPHELES[1]

By F. E. Baisas

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THREE PLATES AND SEVEN TEXT FIGURES

The present paper is one of a series of studies the malaria division of the Philippine Health Service is undertaking in an effort to bring the Philippine anophelines to a standard method of classification. We find that the Philippine species do not conform exactly in all details with the various descriptions given by different authors on Oriental anopheles. We are handicapped, moreover, by the lack of materials from other countries, with which to compare our own.

The specimens dealt with in this study were collected in and around Calauan, Laguna, Luzon, from the latter part of May to the earlier part of August, 1929, and in and around Tungcong Manga, San Jose, Bulacan, Luzon, during September and October of the same year. This period covered the last part of the dry season and almost the whole of the rainy season. The mounted adults as well as their larval and pupal skins, which were mounted in Gater's fluid,[2] are deposited in the Philippine Health Service, Manila.

Grateful acknowledgment is hereby expressed to Dr. C. Manalang, my immediate chief, for valuable suggestions, for correcting and editing this paper, and for the benefit of his wide experience; and to Dr. W. V. King, of the United States Bureau of Entomology, under whom the whole technic and systematic isolation and follow-up of individual insects from larva to adult have been learned and are here employed. Thanks are also due to Dr. G. Villanueva, of malaria-control unit No. 1, and to his men, Messrs. M. Serrano and A. Bagabaldo, for laboratory help and field collections. I also owe a great deal to the field laboratory personnel at Tungcong Manga for company and help. I also thank Dr. Paul F. Russell, of the Rockefeller Foundation, for the free access given me to his personal library and for his kindness in reading this manuscript.

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[1] From the field laboratory, division of malaria control, Philippine Health Service, Tungcong Manga, San Jose, Bulacan Province, Luzon. Submitted for publication November, 1929.

[2] Bull. Ent. Res. 19 pt. 4 (1929) 367–368.

REVIEW OF LITERATURE

There seems to be a general agreement among authors relative to *Anopheles barbirostris* van der Wulp. In the Philippines no other species has been mistaken for it or a synonym credited under this *Anopheles.* Ludlow[3] was the first to report the existence of this mosquito in the Archipelago, followed by Banks,[4] Bezzi,[5] Barber and Walker,[6] Dyar,[7] and Tiedeman.[8] S. R. Christophers[9] could not ascertain from the specimens in the British Museum whether Walker's *vanus* was *barbirostris* or *bancrofti.* "If shewn with certainty to be either," he says, "it would have precedence." N. H. Swellengrebel and J. M. H. Swellengrebel de Graaf[10] reported a variety of *barbirostris* (var. *pallidus*) in Netherlands India, which was later recorded as a distinct species under the name *barbumbrosus* by Strickland.[11] Both *pallidus* and *barbumbrosus* as described by Swellengrebel and Swellengrebel de Graaf[12] and by Strickland and Chowdhury,[13] respectively, differ from the type form (that is, *barbirostris*) only in that the outer anterior clypeal hair has fewer branches (eleven to twenty-two as against sixty or more of the type) than that of typical *barbirostris.* The smallest number of twigs in our series was of those collected with *leucosphyrus* Donitz in a mountain creek at the College of Agriculture, near Los Baños. They vary from twenty to twenty-five or more. Examination of the mesosome and its leaflets showed this kind to be identical with *barbirostris.* In the matter of the number of branches, therefore, of the outer anterior clypeal hair of *barbirostris* in our collections, the range of twenty to forty or an average of thirty each does not fall quite as low as those of *barbumbrosus* or as high as those of *barbirostris* in the countries represented by the works of the above-cited authors. Christophers [14] considers *pallidus* (and, for that matter, probably *barbumbrosus,* which is not mentioned in his provisional list) synonymous with *bancrofti.*

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[3] Can. Entom. 37 (1905) 135.

[4] Philip. Journ. Sci. 1 (1906) 981.

[5] Philip. Journ. Sci. § D 8 (1913) 306.

[6] Philip. Journ. Sci. § B 9 (1914) 389.

[7] Insecutor Inscitiae Menstruus 8 Nos. 10–12 (1920) 184–186.

[8] Journ. Prev. Med. 3 (1927) 227.

[9] Prov. List and Ref. Cat. of the Anoph. (1924) 32.

[10] Bull. Ent. Res. 11 (1920) 82.

[11] As cited by Chowdhury in Ind. Journ. Med. Res. 16 No. 4 (1929) 988.

[12] Bull. Ent. Res. 11 (1920) 82.

[13] The Anoph. Larvae of India, Ceylon and Malaya (1927) 32, pl. V. D.

In a recent publication,[15] Chowdhury described a "new variety of proto-anopheline *A. barbirostris* v. d. Wulp var. *ahomi* found in Upper Assam." Judging from his description and the illustration of the head of the larva, his *ahomi* falls well within what we are calling *A. pseudobarbirostris* Ludlow, in this paper. He says, "Clypeal hairs:—anterior internal long, straight, and has several branches on its distal half; anterior external dendriform branching with more than 50 to 60 twigs; posterior short, straight and usually bifid, but simple and trifid hairs have also been found." Unfortunately, he does not give a full description of the adult,[16] and even the lateral hairs and Martini's hair No. 5 of the sixth abdominal segment, and the stigmal plate of the larva have not been discussed, so we are left to suppose that *ahomi, pseudobarbirostris,* and *bancrofti* are varieties of one species, if not actually the same species.[17]

Christophers has this to say of *bancrofti* larva: ". . . outer anterior clypeal hair formed of about 17 branches only," and of its adult, ". . . legs flecked with scattered white scales; white scales present distal to black ventral tuft; center of mesepimeron devoid of white scales." The points cited for the adult agree with those of our *pseudobarbirostris,* but the outer anterior clypeal hairs of all the larvæ from which they were bred out had numerous close set branches running up to sixty or more each (see fig. 1, *c*). In fact this species can be distinguished from *barbirostris* or the varieties of *hyrcanus* in the larval form by the peculiar pattern of this hair. *Anopheles bancrofti* has been differentiated from *barbirostris* by Giles [18] in the following man-

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[14] Prov. List and Ref. Cat. Anoph. (1924) 32.

[15] Ind. Journ. Med. Res. 16 No. 4 (1929) 988.

[16] He merely mentioned that the male genitalia of *ahomi* showed characters similar to those of typical *barbirostris,* except in the thecal leaflets, which are rather longer and more regular in arrangement on the thecal knob than in the type species.

[17] Prov. List and Ref. Cat. of the Anoph. (1924) 32. F. W. Edwards, Bull. Ent. Res. 14 (1924) 353, says: "*A. pseudobarbirostris* Ludlow from the Philippines has legs similar to those of *A. bancrofti* and also agrees in hypopygeal structure." It seems justifiable, however, to retain *pseudobarbirostris,* provisionally, because of the difference in the larval forms.

[18] Handb. of Gnats or Mosq. ed. 2 (1902) 511.

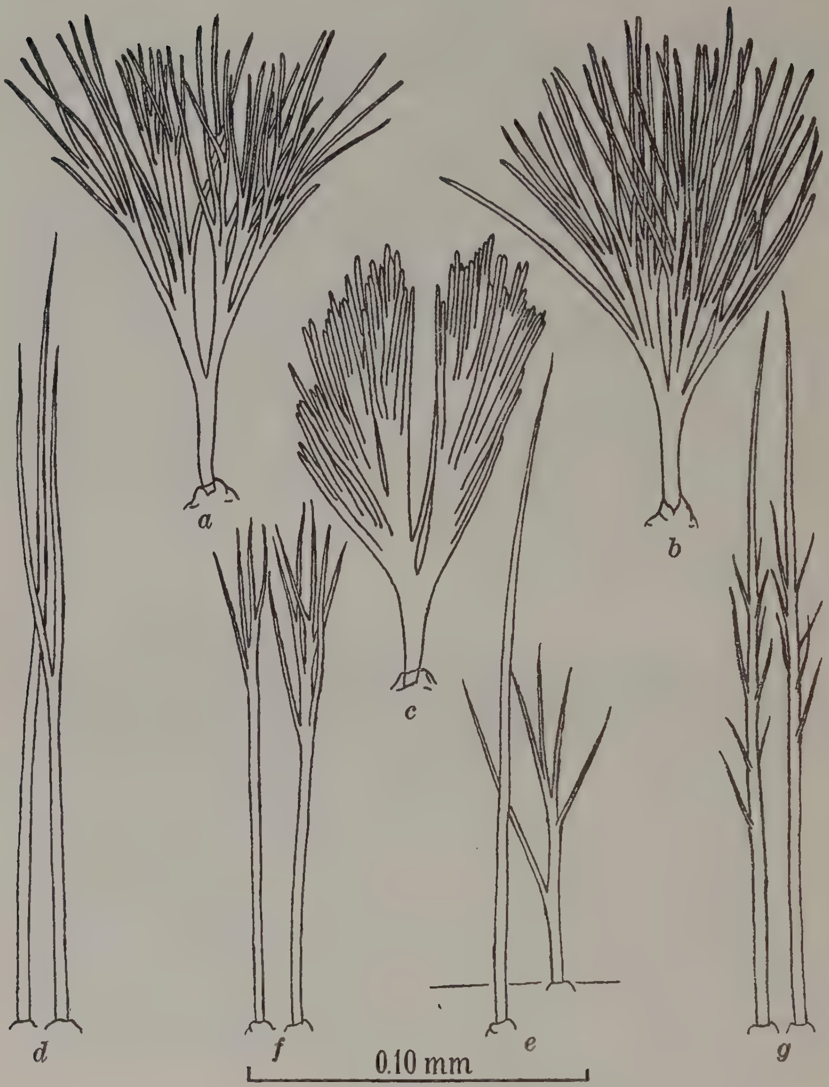

FIG. 1. Anterior clypeal hairs in *Anopheles; a*, *Anopheles barbirostris* van der Wulp, outer anterior clypeal hair; *b*, *Anopheles hyrcanus* var. *sinensis* Wiedeman, outer anterior clypeal hair; *c*, *Anopheles pseudobarbirostris* Ludlow, outer anterior clypeal hair; *d*, *Anopheles barbirostris* van der Wulp, inner anterior clypeal hairs; *e*, *Anopheles barbirostris* van der Wulp, inner anterior clypeal hairs (unusual); *f*, *Anopheles hyrcanus* var. *sinensis*, inner anterior clypeal hairs (uncommon); *g*, *Anopheles pseudobarbirostris* Ludlow, inner anterior clypeal hairs.

ner: "Much resembles *A. barbirostris* v. d. Wulp, but differs in the inner part of the wing being altogether darker and more densely scaled; in the second pale spot being absolutely on the apex in place of sub-apical; and in the banding of the tarsi being much less obvious. In reality, the form is, I think, more nearly allied to *atratipes* Skuse." The wing markings of Philippine *barbirostris* and *pseudobarbirostris* show a general likeness, and vary on similar points; while the rings of the fore and hind tarsi have about the same average measurement. *Anopheles pseudobarbirostris* shows the apical and basal rings of the fourth, and the basal ring of the last tarsi of the hind legs in a much greater frequency and more marked than those of *barbirostris*.

Carter [19] also used *pseudobarbirostris* in naming two specimens (the only ones he has), the larvæ of which he did not see.

Heretofore, we used *hyrcanus* Pallas for *hyrcanus* var. *sinensis*. W. D. v. Tiedeman [20] and others [21] used the latter. Dr. W. V. King also believed that *sinensis* was correct, so we shall employ that designation in this paper.

Carter seems to take *sinensis* as a synonym of *hyrcanus*.[22] James and Liston retracted what they named *hyrcanus* [23] var. *nigerrimus* (not *nigerrimus* Giles), and sunk it under *sinensis* Wiedeman, to which Theobald acceded. *Anopheles nigerrimus* Giles is said to differ from *sinensis* only in having black-tipped palpi. Giles was apparently willing (so said James and Liston)[24] to regard his *nigerrimus* as a specimen of *sinensis*, but Theobald opposed this and treated it as a distinct species. No other specimen of this kind has been found, and James and Liston asserted that either Giles wrongly described his specimen or that it was an extremely rare variety.

Christophers provisionally placed *sinensis* Wiedeman and *nigerrimus* Giles as the Oriental varieties [25] of *hyrcanus*. Borel's [26] illustration of his *nigerrimus* Giles has the palpi white-tipped and so should be regarded as *sinensis* Wiedeman. His description, as very kindly translated by Dr. L. Lopez-Rizal, does not mention whether or not this organ is white-tipped. Some workers placed *vanus* Walker (considered either *barbirostris* or *bancrofti* by Christophers,[27]) among the varieties of *hyrcanus*.[28]

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[19] The Anoph. Mosq. of Ceylon, pt. 1 (1924) 31–32.

[20] Journ. Prev. Med. 3 (1927) 227.

[21] See footnotes 3, 4, 5, and 6.

[22] The Anoph. Mosq. of Ceylon, pt. 1 (1924) 31.

[23] The Anoph. Mosq. of India, ed. 2 (1911) 15.

[24] Loc. cit.

[25] His statement on page 29 is apparently in conflict with that on page 86 (see footnote 9). It is presumed that he meant the latter.

[26] Archives des Inst. Pasteur D'Indochine, 29 and pl. 3.

The fringe spot opposite the posterior branch of the fifth wing vein has been found only twice in our *sinensis*. The inner anterior clypeal hairs of *sinensis* larva are pictured with fine branching by both Theobald [29] and Martini.[30] When this hair is branched in the Philippine *sinensis* the twigs are rather coarse as in fig. 1, *f*.

The field laboratory at Tungcong Manga was occupied at an opportune time as it coincided with the breeding season of a certain variety of *hyrcanus* found only in rice fields and in the newly locked La Mesa Dam. This kind was not encountered during the stay in Calauan. Although its adult does not agree with *paeditaeniatus* Leicester [31] it is undoubtedly the Philippine representative of that species. G. E. Brooke [32] sank *paeditaeniatus* under *vanus*. He distinguished *sinensis* by the apical bands of the tibia and the first four tarsals of the hind legs, from *vanus* which has basal bands to the tibia and all the tarsals. In F. W. Edwards's opinion [33] *hyrcanus* should be substituted for *sinensis*. Later he discussed [34] the varieties of this species, stating, among others, that there is a darker variety throughout the Oriental Region, and still another, *pseudopictus*, having somewhat marked, but very variable and sometimes inconspicuous tarsal bandings. The latest suggestions he has made [35] to assist in distinguishing the chief local varieties are as follows:

> I. for *sinensis*.
>
> Hind tarsi with narrow pale rings at apices of segments only. Wings usually paler than other varieties, dark area variable and sometimes suffused, but dark spot near base of fifth vein short, usually dotlike.

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[27] Prov. List and Ref. Cat. of the Anoph. (1924) 32.

[28] Ludlow, Bull. Ent. Res. 19 pt. 4 (1929) 367–368, credited the Philippines with *vanus* Walker and said it closely resembled *sinensis*. James and Liston, The Anoph. Mos. of India, ed. 2 (1911) 15, received specimens named *vanus* Walker by other workers, which they subsequently renamed *sinensis* Wiedeman.

[29] A Monograph of the Culicidae of the World 3: 18.

[30] Zool. Jahrb. Abeit. fur Sust. Geog. un Bio. der Tiere 46 (1922–23) 539.

[31] The Culicidae of Malaya F. M. S. (1908) 31.

[32] The Identification of Malayan Culicidae (1921) 49.

[33] Bull. Ent. Res. 10 (1920) 129.

[34] Bull. Ent. Res. 12 (1922) 275.

[35] Bull. Ent. Res. 20 (1929) 324.

II. for *nigerrimus* Giles (*paeditaeniatus* Leicester).
Rings of hind tarsi broader, the fourth segment more or less conspicuously white at base as well as at the tip. General color of body much blacker than in other varieties.

The material from which Edwards drew his conclusions included some specimens from the Philippines. Obviously, the black-tipped palpi of Giles's *nigerrimus* is not considered a definite characteristic by him. However, he said that if Leicester's assertion that *paeditaeniatus* larvæ constantly differed from those of *hyrcanus* (*sinensis*), was correct, the two might be considered distinct species. In this particular case Strickland[36] surmised that there might be a larval form intermediate between *sinensis* and *paeditaeniatus* just as there are imagines having characters intermediate between the two. For this reason he did not give *paeditaeniatus* even a varietal status. Christophers gave it a provisional varietal rank.[37]

The larva of the Philippine variety is so like that of *sinensis* that Strickland's presumption is quite correct, although the adults of the Philippine variety, so far as we have seen, invariably differ from those of *sinensis*. My option is to accept Edwards's opinion. Objection to this may, of course, be raised. There are, for instance, *maculatus* and *karwari*, which are accepted as distinct species, although their larvæ differ only in the mere form of the filaments of the leaflets of the abdominal palmate, which difference is sometimes not at all obvious, etc. Still, Edwards's opinion seems to be the least confusing.

DESCRIPTION OF SPECIMENS

THE LARVÆ

In Calauan, Laguna, *Anopheles barbirostris, pseudobarbirostris,* and *hyrcanus* var. *sinensis* were found breeding together in only two places; namely, a large impounded spring full of aquatic vegetation, and a small lake more thickly vegetated. They were associated with numerous larvæ of *A. philippinensis* Ludlow, more numerous (in the former place only) *minimus* Theobald, a few *subpictus* Grassi, and still fewer *A. fuliginosus* Giles. *Anopheles pseudobarbirostris* and *sinensis* were present in good numbers, but *barbirostris* was rather scarce. On the other hand, *barbirostris* was plentiful in rivers and streams and in a well, wherein bred none of the other two. Instead it was found in company with *minimus,* and in one mountain creek with *A. leucosphyrus* Donitz. In Tungcong Manga, *hyrcanus* var. *nigerrimus* was found associated with many *fuliginosus* and *philippinensis* and some *barbirostris* larvæ in rice fields, and only with *fuliginosus* in La Mesa Dam. No *sinensis* or *pseudobarbirostris* was found in any of the breeding places around Tungcong Manga and its vicinity.

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[36] Bull. Ent. Res. 4 (1914) 135.

[37] Prov. List and Ref. Cat. of the Anoph. (1924) 29.

The three distinct species are not difficult to distinguish. In fact more or less accuracy can be attained even with the naked eyes alone, although this method needs some practice and is not recommended for beginners. It must be admitted, however, that *sinensis* and *nigerrimus* would be hard to differentiate if they happened to breed together. The inner anterior submedian thoracic hair of *nigerrimus,* when having only two divisions, has the forking point usually at the middle or subbasally and sometimes subapically; and when branched into three, the branches form a characteristic tripodal arrangement (see fig. 2, *g*).[38] Actual measurement shows that it tends to be shorter than that of *sinensis,* in spite of the fact that its larva generally is much larger than that of the other. The measurement of the antennæ is also of some help. The antenna of the largest *sinensis* larva is only 0.28 millimeter long, while that of the smallest *nigerrimus* measures 0.30 millimeter. The usual antennal length of the former is 0.25 millimeter, and of the latter, 0.32 millimeter. Such differences, depending upon micrometric measurement, are, I think, of doubtful value, and hard to apply in routine work when hundreds of actively moving larvæ are to be examined.

Anopheles barbirostris.—It has long been established by workers in other countries that the principal key in differentiating the larva of *barbirostris* from that of *hyrcanus* and its varieties is the branching of the inner anterior submedian thoracic hairs. We have this in our *barbirostris* collections varying in number from four (see fig. 2, *b*) to fifteen (fig. 2, *h,* is an example of one having branches above ten in number). Swellengrebel and Swellengrebel de Graaf[39] have this hair branched as low as three in their series. The position of the antennal hair

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[38] It should be remarked in this connection that "basally," "subbasally," etc., are here used with diffidence, there being instances when one term supposed to be confined to one species is also applicable to another. Thus, for instance, there are specimens of *sinensis* having one of the branches close to the base and of *pseudobarbirostris* having only truly apical branches.

[39] Bull. Ent. Res. 11 (1920) 82.

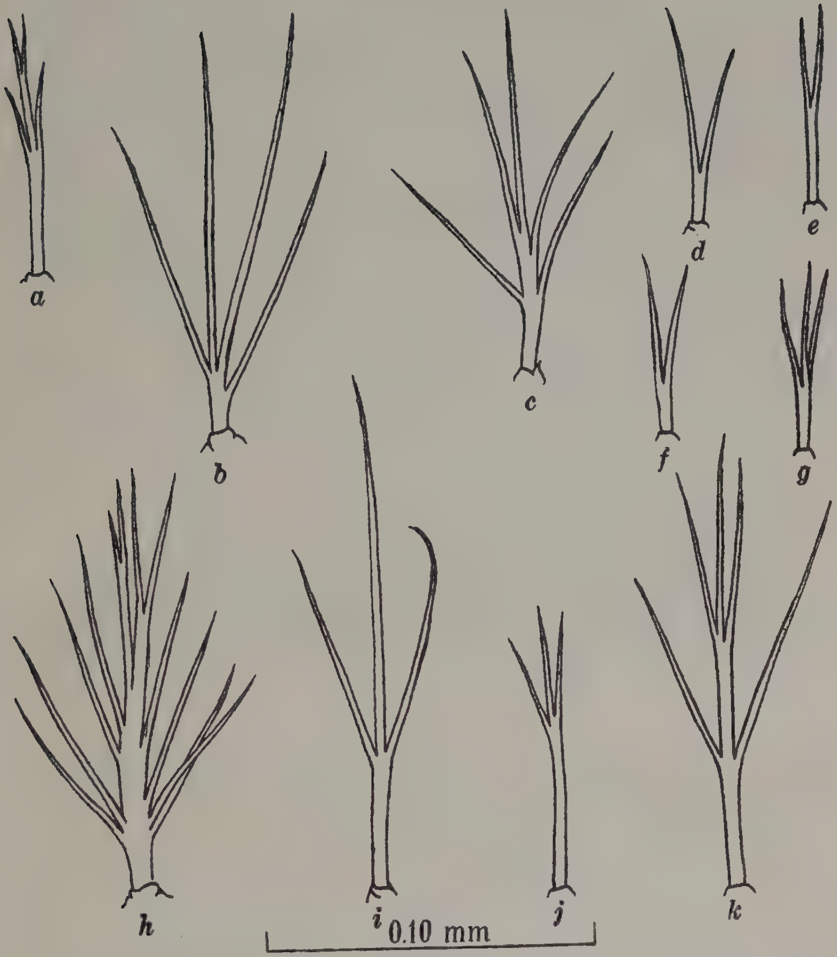

FIG. 2. Inner anterior submedian thoracic hairs in *Anopheles; a* and *j, Anopheles hyrcanus* var. *sinensis* Wiedeman; *b,* and *h, Anopheles barbirostris* van der Wulp; *c, i,* and *k, Anopheles pseudobarbirostris* Ludlow; *d, e, f,* and *g, Anopheles hyrcanus* var. *nigerrimus* Giles.

in *barbirostris* is quite constantly around one-third from the base of the antennæ while those of *pseudobarbirostris* and *hyrcanus* varieties, *sinensis* and *nigerrimus,* are at about the basal half or less. Other characteristics of aid in identification are the following:

(*a*) Inner anterior clypeal hairs simple, or one or the other, very seldom both, forked (see fig. 1, *d*).[40] A peculiar abnormality rarely seen is as shown in fig. 1, *e*.

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[40] The accompanying drawings were all traced with the aid of a camera lucida by the writer.

(*b*) Outer anterior clypeal branched as in fig. 1, *a*, very often less in number than that of either *hyrcanus* varieties or *pseudobarbirostris*.
(*c*) Postclypeal hairs usually branched, the branches being closely appressed to the main stem and are not truly basal.
(*d*) Inner and outer occipital hairs averaging 10 and 9.6 branches each, respectively.
(*e*) Antennæ ranging from 0.25 to 0.35 millimeter long, with an average of 0.30 millimeter. The antennal hair has fewer branches, eight on the average, than that of *pseudobarbirostris* or of *sinensis*.
(*f*) Inner anterior submedian thoracic as above stated.
(*g*) Antepalmate hairs[41] of the fourth and fifth abdominal segments usually forked at the middle (see fig. 3, *b*).
(*h*) Lateral hairs of the sixth abdominal segment very short, less than half the length of those on the preceding two segments (fig. 3, left side, *a*).
(*i*) Hair No. 5 (Martini's) present on the sixth abdominal segment (fig. 3, left side, *d*).
(*j*) No club-shaped projection between the stigmata (fig. 5, *c*).

Anopheles pseudobarbirostris.—Under the microscope, the larva of *A. pseudobarbirostris* Ludlow has very definite distinguishing characteristics which render it easily recognized. These may be summed up as follows:

(*a*) Inner anterior clypeal hairs finely branched (fig. 1, *g*).
(*b*) Outer anterior clypeal hairs with numerous close-set branches as in fig. 1, *c*.
(*c*) Postclypeal short, usually simple.
(*d*) Inner and outer occipital hairs averaging 10 and 6.9 branches each, respectively.
(*e*) Antennæ ranging in length from 0.30 to 0.35 millimeter. The antennal hair arises at about the basal half and contains more branches than that of *barbirostris* (average 16.5).
(*f*) Inner anterior submedian thoracic branched at the middle or subapically from two to five, usually only three. It is so very much longer and stouter than that of *sinensis* or *nigerrimus* that although they may be similar in the number and position of branches one cannot be mistaken for the other (see figs. 2, *c*, *i*, *k*).
(*g*) Antepalmate of the fourth and fifth abdominal segments usually branched apically (figs. 3 and 4, right side, *b*).
(*h*) Lateral hairs on the sixth abdominal segment long, developed. On the seventh shorter but also developed (figs. 3 and 4, right side, *a*).
(*i*) Hair No. 5 on segment six absent (figs. 3 and 4, right side).
(*j*) Stigmal club present (fig. 5, *b*).

[41] Russell, P. F., Am. Journ. Hyg. 5 No. 2 (1925) 166.

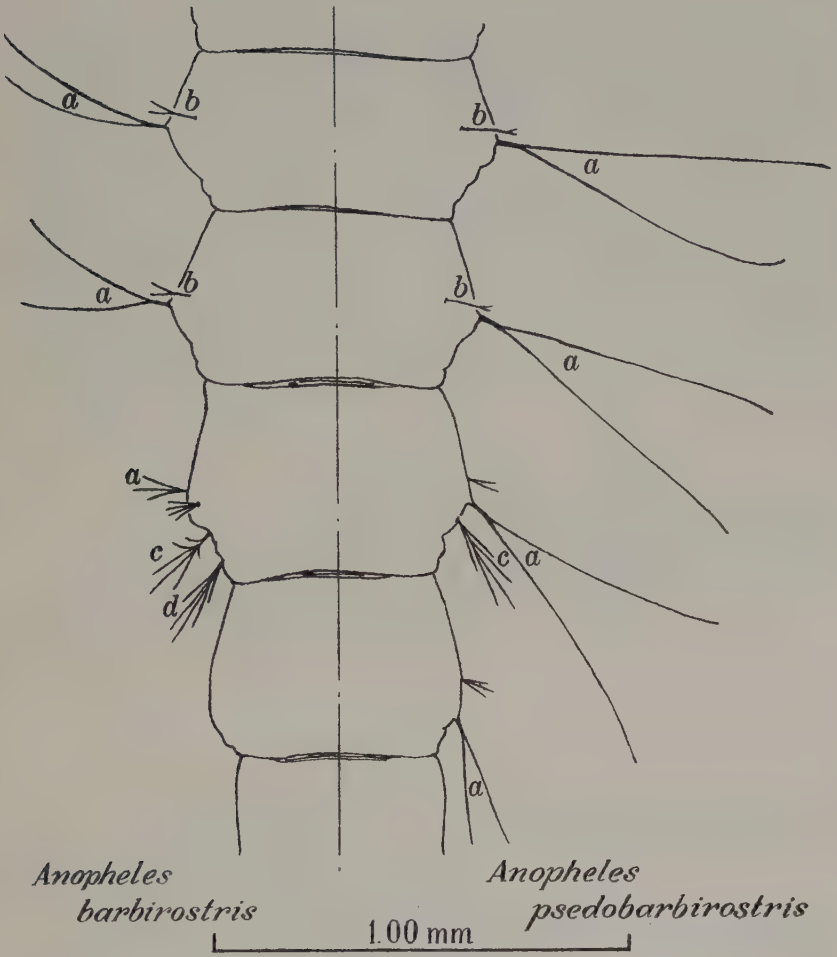

FIG. 3. *Anopheles barbirostris-pseudobarbirostris,* fourth, fifth, sixth, and seventh abdominal segments of larva; *a,* lateral hairs; *b,* antepalmate hairs; *c,* Martini's hair No. 9; *d,* Martini's hair No. 5.

Anopheles hyrcanus var. *sinensis.*—The larva of *A. hyrcanus* var. *sinensis* has—

(*a*) Inner anterior clypeal hairs simple, or one or the other or both forked, or coarsely branched as in fig. 1, *f.*

(*b*) Outer anterior clypeal hairs as shown in fig. 1, *b.*

(*c*) Postclypeal short, almost always branched, the branches originating from the base, except of course, when the branch is rebranched, and are well spread.

(*d*) Inner and outer occipital hairs averaging eight and six branches each, respectively.

(*e*) Length of the antennæ from 0.24 to 0.28 millimeter. The antennal hair arises at less than the basal half of the antennæ. Its branches average 10.5 each.

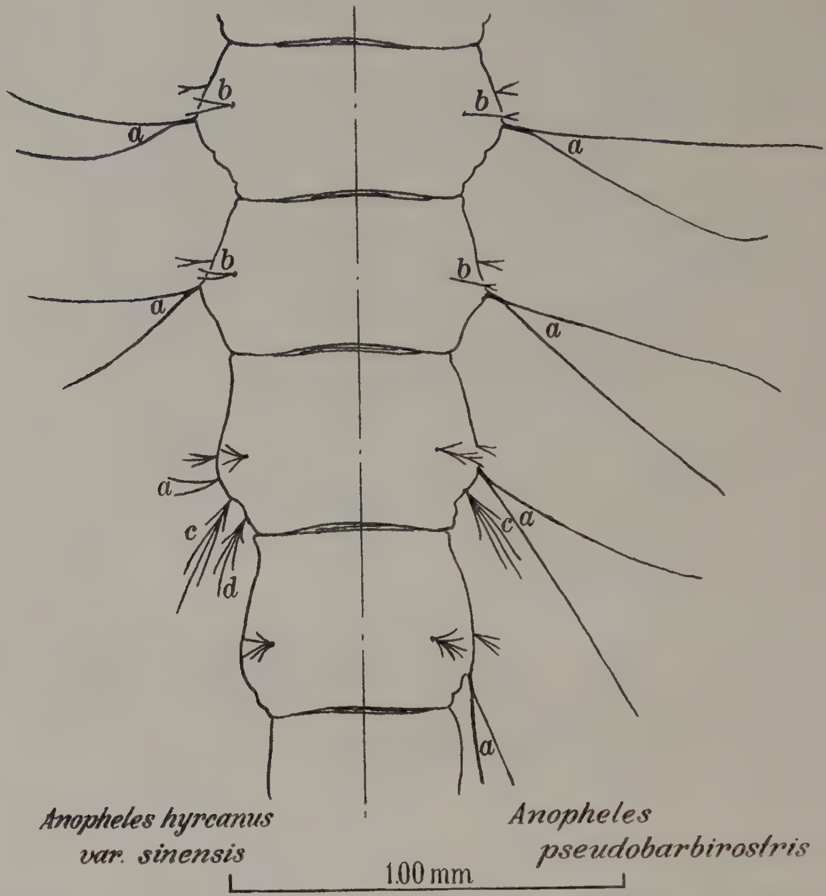

FIG. 4. *Anopheles sinensis-pseudobarbirostris*, showing fourth, fifth, sixth, and seventh abdominal segments; *a*, lateral hairs; *b*, antepalmate hairs; *c*, Martini's hair No. 9; *d*, Martini's hair No. 5.

(*f*) Inner anterior submedian thoracic short, simple or branched apically into usually only two or three. See Fig. 2 *a* and *j*. Generally the middle anterior submedian thoracic has fewer branches than that of either of the other species in this group, while the outer anterior submedian is occasionally bifid.

(*g*) Antepalmate of the fourth and fifth abdominal segments usually branched basally (see fig. 4, left side, *b*).

(*h*) Lateral hairs of the sixth abdominal segment short as of *barbirostris;* on the seventh absent.

(*i*) Hair No. 5 on the sixth abdominal segment present.

(*j*) No stigmal club (fig. 5, *a*).

In addition to the above some recognize a difference in shape and serrations of the leaflets of the abdominal palmate hairs, and in the number of the long and short teeth of the lateral combs (refer to figs. 5, *e*, and 6, *a*). In this connection, it may be stated that the leaflets of the abdominal palmate of *barbirostris* show a more-marked variation than could be seen in any other species (see figs. 5, *g* and *h*).

Anopheles hyrcanus var. *nigerrimus.*—As stated above, the larva of *nigerrimus* differs so slightly, if at all, from *sinensis* that it would be extremely difficult if not impossible to tell which is which if they were together. They seem to breed separately, however, so that it would perhaps be better to differentiate them by means of the types of breeding places. For further details relative to the larva of this species, the reader is referred to Table 1.

THE ADULTS

Of the 167 *Anopheles barbirostris*, 110 *pseudobarbirostris*, 133 *hyrcanus* var. *sinensis*, and 168 *hyrcanus* var. *nigerrimus* larvæ isolated individually after examination, 108, 83, 96, and 111, respectively, reached the adult stage. They came out as follows:

	Males.	Females.
Anopheles barbirostris	43	65
Anopheles pseudobarbirostris	36	47
Anopheles hyrcanus var. *sinensis*	51	45
Anopheles hyrcanus var. *nigerrimus*	55	56

The rest of the larvæ died, some in the pupal stage, or else were discarded after staying in the isolation jars without apparent development for some days.

Detailed descriptions of these species in the adult forms can be found in various works,[42] so only the more important points need be recapitulated here, as follows:

Anopheles barbirostris van der Wulp.

(*a*) Shaggy palpi entirely black (Plate 1, fig. 3).

(*b*) Subcostal spot[43] of the wing variable in size and may as well as not involve the anterior, and sometimes also the posterior, portion of the first longitudinal vein.

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[42] Anoph. Mosq. of Ceylon (1924), for *barbirostris*, *pseudobarbirostris*, and *hyrcanus*, pages 30–32; also pl. 15. Anoph. Mosq. of India ed. 2 (1911), for *barbirostris* and *sinensis*; also full figures of same. Handb. of Gnats or Mosq. ed. 2 (1902) 511, for *bancrofti*; page 308 for *barbirostris* and *sinensis*. Bull. Ent. Res. 12 (1922) 50, for *pseudobarbirostris*; pages 48–49 for *sinensis*. See also Stanton, Notes on Malayan Culicidae, page 69, for *barbirostris*; page 75 for *sinensis*.

[43] Root's pilotaxy of the wing is used here. See his studies on Brazilian mosquitoes, Am. Journ. Hyg. 6 (1926) 691 and pl. 1.

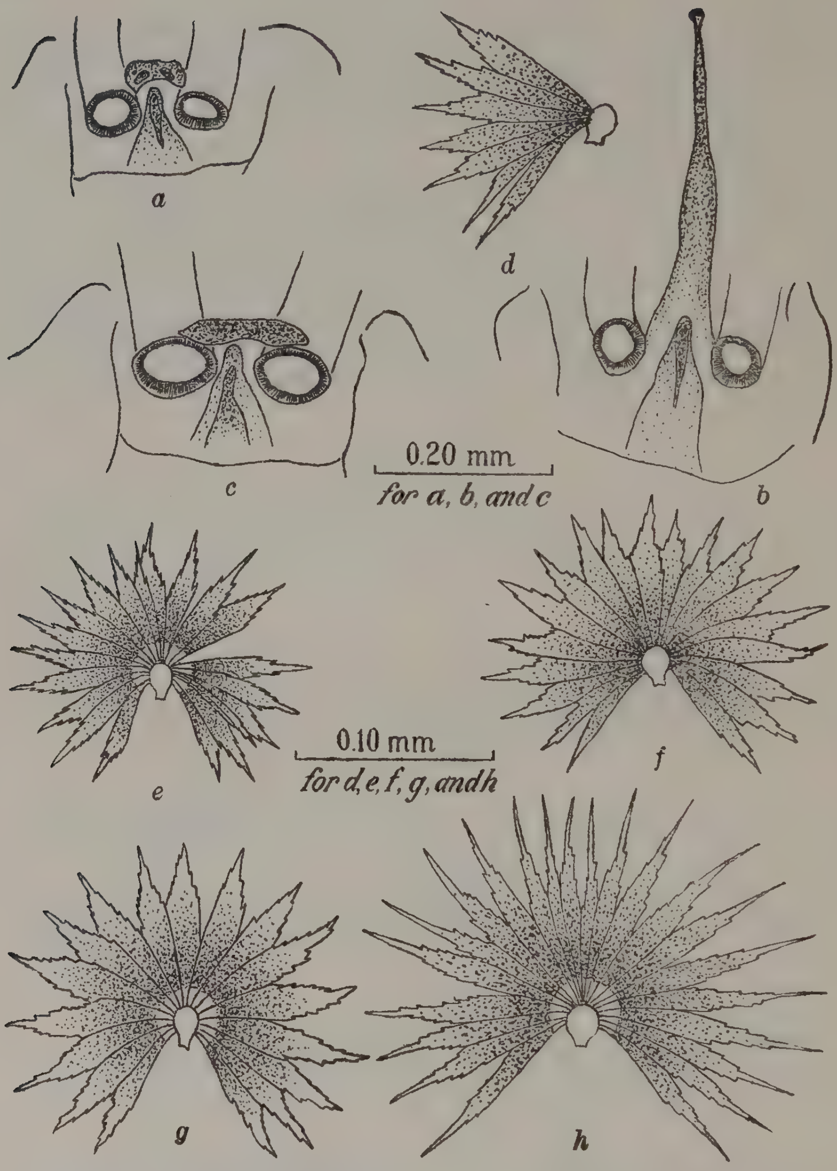

FIG. 5. Stigmal plate and palmate hair of the fourth abdominal segment in various species of *Anopheles; a, Anopheles hyrcanus* var. *sinensis* larva, stigmal plate; *b, Anopheles pseudobarbirostris* larva, stigmal plate, showing club; *c, Anopheles barbirostris* larva, stigmal plate; *d, Anopheles hyrcanus* var. *nigerrimus*, part of a palmate hair of the fourth abdominal segment; ***e, Anopheles hyrcanus*** **var. *sinensis*, palmate** hair of the fourth abdominal segment; *f,* ***Anopheles pseudobarbirostris*, palmate hair of the fourth abdominal segment**; *g* and *h*, ***Anopheles barbirostris*, two types of palmate hairs of the fourth abdominal segment.**

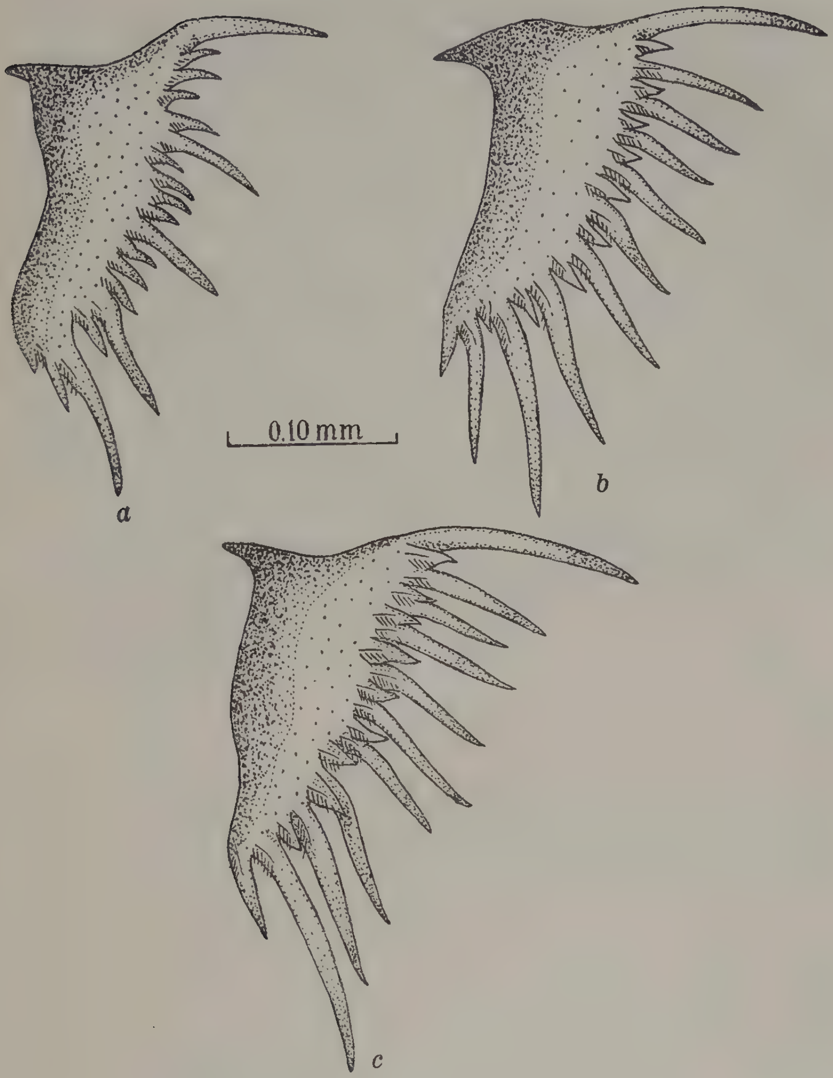

FIG. 6. Lateral comb in *Anopheles*; *a*, *Anopheles hyrcanus* var. *sinensis*; *b*, *Anopheles pseudobarbirostris*; *c*, *Anopheles barbirostris*.

(*c*) There is usually one (at times none) basal costal spot.

(*d*) The apical spot has usually a black interruption at its middle, which may at times be wanting, or else joined with the black rim toward the apex.

(*e*) The next spot is opposite the third longitudinal but may be as wide as to involve also the area opposite 4.1.

(*f*) The fringe spot opposite 5.2 is variable in size and may sometimes be entirely wanting.

TABLE 1.—*Comparative table of larval hairs in two species and two varieties of Anopheles.*

Character.	*Anopheles barbirostris.*		*Anopheles pseudobarbirostris.*		*Anopheles hyrcanus* var. *sinensis.*		*Anopheles hyrcanus* var. *nigerrimus.*	
		Per cent.		*Per cent.*		*Per cent.*		*Per cent.*
Inner anterior clypeal hairs.								
Simple	116	74	0	0	45	37	154	92
One or both branched	40	26	110	100	75	63	12	8
Outer anterior clypeal hairs.								
Number of branches	20–40		47–63		31–42		28–76	
Average number of branches	30		54		37		45	
Postclypeal hairs.								
Simple	5	8	77	86	1	2	2–8	
One or both branched	52	92	12	14	55	98	120	100
Inner occipital hairs.								
Number of branches	5–16		6–13		6–12		3–10	
Average number of branches	10		10.4		8.3		6	
Outer occipital hairs.								
Number of branches	6–15		2–11		3–11		2–8	
Average number of branches	9.6		6.9		6.0		5.0	
Antenna.								
Range of length mm	0.25–0.35		[a]0.30–0.35		0.24–0.28		[b]0.30–0.35	
Average length mm	0.30		0.32		0.25		0.32	
Antennal hair.								
Number of branches	4–24		11–24		6–15		5–8	
Average number of branches	8		16.5		10.5		6.0	
Average position of antennal hair from the base of the antenna	⅓		½ or <		½ or <		< ½	
Inner anterior submedian thoracic hair.								
Number of branches	4–15		1–6		28	21	81	48
Average number of branches	8		3.0		104	79	86	52
Middle anterior submedian thoracic hair.								
Number of branches	9–19		8–17		6–13		9–19	
Average number of branches	14		12.8		10.0		14.0	
Lateral hairs of sixth abdominal segment	Short in all		Long in all		Short in all		Short in all.	
Lateral hairs of seventh abdominal segment	Absent in all		Present in all		Absent in all		Absent in all.	
Hair 5 of sixth abdominal segment	Present in all		Absent in all		Present in all		Present in all.	
Stigmal club	Absent in all		Present in all		Absent in all		Absent in all.	

[a] In one specimen the antenna measured 0.28 millimeter only.

[b] In one specimen the antenna measured 0.29 millimeter only.

(*g*) Scattered black scales very often present basal to the second black lengths from the apex of the sixth longitudinal vein (see Plate 2, figs. 1, 2, and 3).

(*h*) Its legs, described as "sooty" by Giles, "inconspicuously spotted" by Carter,[44] and "dark brown to black" by James and Liston,[45] appear to me to have very widely scattered brownish or dull whitish narrow scales which are by no means as conspicuous or pronounced as those in *pseudobarbirostris* (see Plate 1, figs. 9 and 10).

(*i*) Mesepimeral white scales present. Some white scales may also be present on the ventral side of the abdomen but the ventral segmental white scales as shown in Rodenwaldt's chart[46] is not a constant characteristic. This particular point is usual in *pseudobarbirostris*. The black ventral tuft of the female abdomen has white scales on its cephalic side (see fig. 7, *h*).

(*j*) Male mesosome long with long serrated leaflets (see fig. 7, *e*).

Anopheles pseudobarbirostris.

(*a*) Unbanded, shaggy palpi (see Plate 1, fig. 4).

(*b*) Subcostal spot similar to and as variable as that of *barbirostris.*

(*c*) There may be one or two basal costal spots.

(*d*) Similarly, the apical spot may or may not have the black interruption at the middle.

(*e*) The next spot is opposite 4.1.

(*f*) The fringe spot opposite 5.2 is variable in size and may also be absent.

(*g*) Scattered black scales basal to second black length from the apex of the sixth longitudinal present (see, Plate 2, fig. 4, and Plate 3, fig. 1).

(*h*) The legs are described as "flecked with scattered white scales" by Christophers[47] and "well sprinkled with white scales" by Ludlow.[48] These white scales are easily detected under the microscope particularly on the ventral aspect of the fore femur where the white scales predominate over the black ones (see Plate 1, figs. 11, 12, and 13).

(*i*) Mesepimeral white scales absent, but clumps of them elsewhere on the pleura and coxæ show conspicuously. There are also white scales cephalad and caudad to the female black ventral tuft (refer to fig. 7, *f*), and a symmetrically arranged group of white ones, three or more in a group in females, less in males, on every segment, except the first.

(*j*) Mesosome of the male long with long unserrated leaflets (fig. 7, *c*).

[44] Anoph. Mosq. of Ceylon, pt. 1 (1924) 31–32.

[45] Anoph. Mosq. of India, ed 2 (1911) 15.

[46] De Anoph. van Nether. Oost-Indie.

[47] Prov. List and Ref. Cat. Anoph. (1924) 32.

[48] U. S. Army Bull. Office of the Surg. Gen. No. 4 (November, 1913) 53.

Anopheles hyrcanus var. *sinensis.*

(*a*) Palpi white banded. These bands are so variable in breadth that a number of types could be built from them if what seems to be indicated by Christophers[49] is followed. He recognizes a difference in breadth of the third white band from the apex in relation to the apical and subapical bands between *hyrcanus* and *sinensis*. Ours have theirs usually narrower than the apical or subapical but many be wider. The queerest of them all, however, is when the apical and subapical join together to form only one (Plate 1, figs. 1 and 2; see Table 2).

(*b*) The subcostal spot is almost always wide and involves the anterior as well as the posterior portion of the first longitudinal.

(*c*) Basal costal spot absent.

(*d*) The apical spot is continuous without any black interruption at the middle.

(*e*) The next spot extends sometimes from opposite 2.1 to midway between 3 and 4.1.

(*f*) Fringe spot opposite 5.2 rarely present.

(*g*) Usually there are no scattered black scales basal to the second black length from the apex of the sixth longitudinal vein, and the two black lengths of this vein may join together to form a long one (Plate 3, fig. 2).

(*h*) Legs without scattered white scales (Plate 1, figs. 7 and 8).

(*i*) No mesepimeral white scales and very scanty if at all elsewhere on the pleura or coxæ.

(*j*) Mesosome short with short leaflets, both sides of which are unevenly serrated (fig. 7, *a* and *d*).

Anopheles hyrcanus var. *nigerrimus* differs so markedly from var. *sinensis* in the very wide bandings of the fore and hind tarsi that it can be determined even with the unaided eyes. Microscopical measurements show that its palpal bands are somewhat wider, particularly the apical, than those of *sinensis*. The next bands, however, are usually so indefinite and incomplete (the third may be entirely absent) that they may as well not be called bands at all. Moreover, under certain lights even the black scales appear silvery, thus making the definition of the bands more vague. Like those of *sinensis* the apical and subapical bands may be fused to form one. The wing appears darker than that of *sinensis*, due to the smallness of the subcostal and apical spots, and the absence of the pale interruption involving both the subcosta at about one-third from the base, and the point adjacent to it of the first longitudinal. There are also no other yellow interruptions elsewhere in the first longitudinal except near its apex, while the second and fourth have continuous black toward the base. The fringe spot at the tip of the wing extends from opposite the first longitudinal to almost 4.1 in some instances, while the fringe spot opposite 5.2 has not been seen in any of our specimens (compare Plate 3, figs. 2, 3, and 4; see Table 3).

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[49] Prov. List and Ref. Cat. Anoph. (1924) 32.

The first three tarsi of the fore legs of *nigerrimus* have very wide apical white bands. Exception of the first tarsi has to be made, sometimes, as its minimum is even lower than that of *sinensis* (0.03 to 0.05 millimeter) although it has a maximum measurement of 0.28 to 0.16 millimeter of *sinensis*. The second has the same exception to the rule so that only the third can be used with certainty (refer to Table 2, and Plate 1, figs. 5 and 6). The second, third, and fourth hind tarsi show very definite differences from those of *sinensis* as can be seen by referring to Table 4.

TABLE 2.—*Comparative table of palpal bands in two varieties of Anopheles hyrcanus.*

Counting from the tip.	Band.					
	First.		Second.		Third.	
	Anopheles hyrcanus var. *sinensis*.	*Anopheles hyrcanus* var. *nigerrimus*.	*Anopheles hyrcanus* var. *sinensis*.	*Anopheles hyrcanus* var. *nigerrimus*.	*Anopheles hyrcanus* var. *sinensis*.	*Anopheles hyrcanus* var. *nigerrimus*.
None	0	0	0	0	0	2
Mere suggestion	0	0	0	0	0	20
0.02 mm occurs	4	0	0	0	0	0
0.05 mm occurs	23	2	2	19	7	24
0.08 mm occurs	10	8	27	26	29	10
0.10 mm occurs	1	28	8	5	1	1
0.12 mm occurs	0	6	0	0	0	0
0.15 mm occurs	0	6	0	0	0	0
Average width mm	0.05	0.10	0.08	0.07	0.07	(a)

Band.	Times.	
First = second = third	2	0
Second = third	12	15
Third less than second	22	31
Third wider than second	4	1
First and second fused together	2	7

[a] Almost nil.

The thecal leaflets show very few serrations, which are confined only to the outer border of the largest, and sometimes also of the next largest, leaflets (fig. 7, *b*).

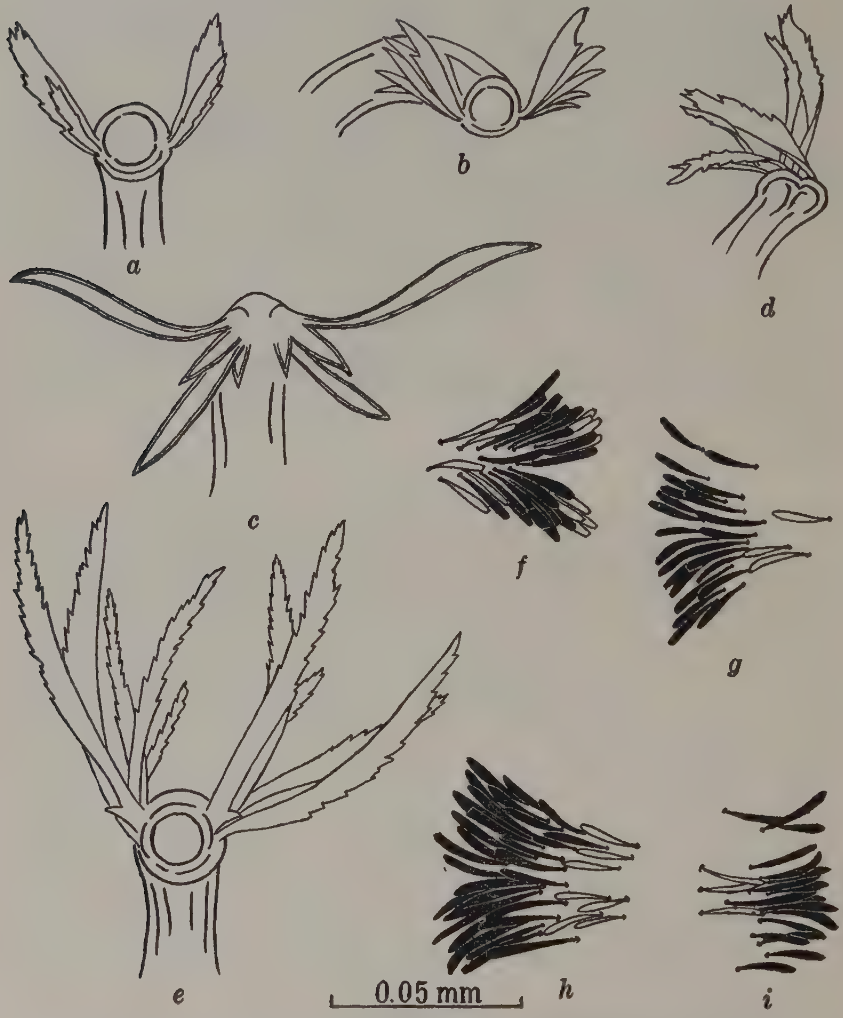

FIG. 7. Mesosome and scale tuft in various species of *Anopheles; a, Anopheles hyrcanus* var. *sinensis*, distal part of mesosome and its leaflets; *b, Anopheles hyrcanus* var. *nigerrimus*, distal portion of mesosome and its leaflets; *c, Anopheles pseudobarbirostris*, distal portion of mesosome and its leaflets; *d, Anopheles hyrcanus* var. *sinensis*, mesosome and leaflets, isolated and pressed under a cover glass; *e, Anopheles barbirostris*, distal portion and leaflets of mesosome; *f, Anopheles pseudobarbirostris*, female, ventral scale tuft of the abdomen; *g, Anopheles hyrcanus* var. *nigerrimus*, female, ventral scale tuft of the abdomen; *h, Anopheles barbirostris*, female, ventral scale tuft of the abdomen; *i, Anopheles hyrcanus* var. *sinensis*, female, ventral scale tuft of the abdomen.

TABLE 3.—*Comparative table of wing spots in two species and two varieties of Anopheles.*

Character.	*Anopheles barbirostris.*		*Anopheles pseudobarbirostris.*		*Anopheles hyrcanus* var. *sinensis.*		*Anopheles hyrcanus* var. *nigerrimus.*	
		Per ct.		*Per ct.*		*Per ct.*		*Per ct.*
Basal costal spot.								
None	21	22.5	13	19	75	100	116	100
One	72	77.5	40	60	0	------	0	------
Two	0	0	14	21	0	------	0	------
Subcostal spot.								
Minimum length mm	0.07	------	0.07	------	0.10	------	(a)	------
Maximum length mm	0.18	------	0.20	------	0.20	------	0.20	------
Average length mm	0.12	------	0.12	------	0.14	------	0.12	------
Commonest mm	0.15	------	0.10	------	0.15	------	0.10	------
Number measured	79	------	67	------	73	------	115	------
Apical spot.								
Black at middle	69	80	61	95	0	------	0	------
Without black	19	20	3	(b)5	------	100	------	100
Total examined	88	------	64	------	72	------	116	------
Fringe spot at wing tip.								
Opposite 3 only	45	50	0	------	0	------	0	------
Opposite 4.1 only	14	16	66	------	0	------	0	------
Opposite 3 to 4.1	31	34	[c] 1	------	0	------	0	------
Opposite 1 to 3	------	------	------	------	0	------	61	54
Opposite 1 to 4.1	------	------	------	------	0	------	3	2.5
Opposite 2.1 to 3	------	------	------	------	72	100	47	42.0
Opposite 2.1 to 4.1	------	------	------	------	0	------	2	1.5
Fringe spot opposite 5.2.								
Present	87	93	64	96	2	3	0	------
Absent	6	7	3	4	73	97	------	100
Total examined	93	------	67	------	75	------	116	------

[a] Mere suggestion. [b] Very faint. [c] Absent.

SUMMARY

For some years the Philippines has been credited with a number of species belonging to the so-called "Myzorhynchus" group (now "Anopheles") of anopheline mosquitoes. The earliest accounts were based on adults caught wild and sent to foreign countries for determination. The present study is an attempt at a systematic treatment of these insects from the larval to the adult stages. As a result, it has been shown that the Philippine species do not exactly tally with the descriptions given for the same species in other countries. The main points of differentiation in both the larval and adult forms of the species studied are here given. The lateral hairs, and hairs Nos. 5 and 9 (Martini's) of the sixth segment, and the stigmal club are used in addition to those commonly employed in the determination of larvæ. A discussion of the available literature as a basis of comparison with the Philippine species is also included.

TABLE 4.—*Comparative table of tarsal rings in two species and two varieties of Anopheles.*

Character.	*Anopheles barbirostris.*	*Anopheles pseudobarbirostris.*	*Anopheles hyrcanus* var. *sinensis.*	*Anopheles hyrcanus* var. *nigerrimus.*
Fore tarsi; first ring.				
Minimum mm	0.08	0.05	0.05	0.03
Maximum mm	0.14	0.10	0.16	0.28
Average mm	0.10	0.07	0.10	0.11
Commonest mm	0.10	0.07	0.10	0.08
Number measured	62	51	71	114
Fore tarsi; second ring.				
Minimum mm	0.03	0.03	0.07	0.08
Maximum mm	0.10	0.10	0.17	0.47
Average mm	0.08	0.05	0.12	0.24
Commonest mm	0.08	0.05	0.12	0.20
Number measured	62	51	71	115
Fore tarsi; third ring.				
Minimum mm	----------	----------	----------	0.10
Maximum mm	----------	----------	----------	0.35
Average mm	----------	----------	----------	0.18
Commonest mm	----------	----------	----------	0.20
Number measured	----------	----------	----------	115
Hind tarsi; first ring.				
Minimum mm	0.07	0.05	0.02	
Maximum mm	0.10	0.10	0.10	
Average mm	0.09	0.077	0.067	(a)
Commonest mm	0.10	0.10	0.07	
Number measured	43	56	71	
Hind tarsi; second ring.				
Minimum mm	0.07	0.05	0.04	0.08
Maximum mm	0.10	0.10	0.12	0.27
Average mm	0.08	0.07	0.087	0.16
Commonest mm	0.08	0.07	0.10	0.18
Number measured	43	56	71	115
Hind tarsi; third ring.				
Minimum mm	----------	----------	0.05	0.18
Maximum mm	----------	----------	0.12	0.35
Average mm	----------	----------	0.09	0.26
Commonest mm	----------	----------	0.10	0.25
Number measured	----------	----------	71	115
Hind tarsi; fourth basal ring.				
Minimum mm	----------	----------	0.00	0.05
Maximum mm	----------	----------	0.02	0.18
Average mm	----------	----------	----------	0.10
Commonest mm	----------	----------	0.00	0.10
Number measured	----------	----------	61	115
Hind tarsi; fourth apical ring.				
Minimum mm	----------	----------	0.03	0.10
Maximum mm	----------	----------	0.10	0.28
Average mm	----------	----------	0.07	0.17
Commonest mm	----------	----------	0.07	0.15
Number measured	----------	----------	71	115

[a] Insignificant.

ILLUSTRATIONS

PLATE 1

FIG. 1. *Anopheles sinensis*, female palpi, dorsal view.
2. *Anopheles sinensis*, female palpi, dorsal view (uncommon).
3. *Anopheles barbirostris*, female palpi, dorsal view.
4. *Anopheles pseudobarbirostris*, female palpi, dorsal view.
5. *Anopheles hyrcanus* var. *nigerrimus*, fore tarsi.
6. *Anopheles hyrcanus* var. *nigerrimus*, hind tarsi.
7. *Anopheles hyrcanus* var. *sinensis*, fore tarsi.
8. *Anopheles hyrcanus* var. *sinensis*, hind tarsi.
9. *Anopheles barbirostris*, fore tarsi.
10. *Anopheles barbirostris*, hind tarsi.
11. *Anopheles pseudobarbirostris*, fore tarsi.
12. *Anopheles pseudobarbirostris*, hind tarsi.
13. *Anopheles pseudobarbirostris*, portion of hind femur, highly magnified, showing the white and black scales.

PLATE 2

FIG. 1. *Anopheles barbirostris*, female wing, showing a very faint black interruption at the middle of the apical spot.
2. *Anopheles barbirostris*, female wing, showing the absence of the fringe spot opposite the posterior fork of the fifth longitudinal vein.
3. *Anopheles barbirostris*, female wing, showing smaller spots. (Compare with fig. 2.)
4. *Anopheles pseudobarbirostris*, female wing.

PLATE 3

FIG. 1. *Anopheles pseudobarbirostris*, female wing.
2. *Anopheles hyrcanus* var. *sinensis*, female wing, showing the yellow interruption at about the middle of the subcosta, involving also the first longitudinal vein. Note also the size of the subcostal spot compared with those of the following two figures.
3. *Anopheles hyrcanus* var. *nigerrimus*, female wing, showing the smallness of the subcostal and apical spots; also the absence of the yellow spot involving the subcosta and first longitudinal. Note the predominance of black scales on the second and fourth longitudinal veins in contrast with those of *A. sinensis*.
4. *Anopheles hyrcanus* var. *nigerrimus*, female wing, showing only one continuous black length on the sixth longitudinal. This peculiarity also occurs in *A. sinensis*.

TEXT FIGURES

FIG. 1. Anterior clypeal hairs in *Anopheles; a, Anopheles barbirostris* van der Wulp, outer anterior clypeal hair; *b, Anopheles hyrcanus* var. *sinensis* Wiedeman, outer anterior clypeal hair; *c, Anopheles pseudobarbirostris* Ludlow, outer anterior clypeal hair; *d, Anopheles barbirostris* van der Wulp, inner anterior clypeal hairs; *e, Anopheles barbirostris* van der Wulp, inner anterior clypeal hairs (unusual); *f, Anopheles hyrcanus* var. *sinensis*, inner anterior clypeal hairs (uncommon); *g, Anopheles pseudobarbirostris* Ludlow, inner anterior clypeal hairs.

2. Inner anterior submedian thoracic hairs in *Anopheles; a,* and *j, Anopheles hyrcanus* var. *sinensis* Wiedeman; *b,* and *h, Anopheles barbirostris* van der Wulp; *c, i,* and *k, Anopheles pseudobarbirostris* Ludlow; *d, e, f,* and *g, Anopheles hyrcanus* var. *nigerrimus* Giles.

3. *Anopheles barbirostris-pseudobarbirostris,* fourth, fifth, sixth, and seventh abdominal segments of larva; *a,* lateral hairs; *b,* antepalmate hairs; *c,* Martini's hair No. 9; *d,* Martini's hair No. 5.

4. *Anopheles sinensis-pseudobarbirostris,* showing fourth, fifth, sixth, and seventh abdominal segments; *a,* lateral hairs; *b,* antepalmate hairs; *c,* Martini's hair No. 9; *d,* Martini's hair No. 5.

5. Stigmal plate and palmate hair of the fourth abdominal segment in various species of *Anopheles; a, Anopheles hyrcanus* var. *sinensis* larva, stigmal plate; *b, Anopheles pseudobarbirostris* larva, stigmal plate, showing club; *c, Anopheles barbirostris* larva, stigmal plate; *d, Anopheles hyrcanus* var. *nigerrimus,* part of a palmate hair of the fourth abdominal segment; *e, Anopheles hyrcanus* var. *sinensis,* palmate hair of the fourth abdominal segment; *f, Anopheles pseudobarbirostris,* palmate hair of the fourth abdominal segment; *g* and *h, Anopheles barbirostris,* two types of palmate hairs of the fourth abdominal segment.

6. Lateral comb in *Anopheles; a, Anopheles hyrcanus* var. *sinensis; b, Anopheles pseudobarbirostris; c, Anopheles barbirostris.*

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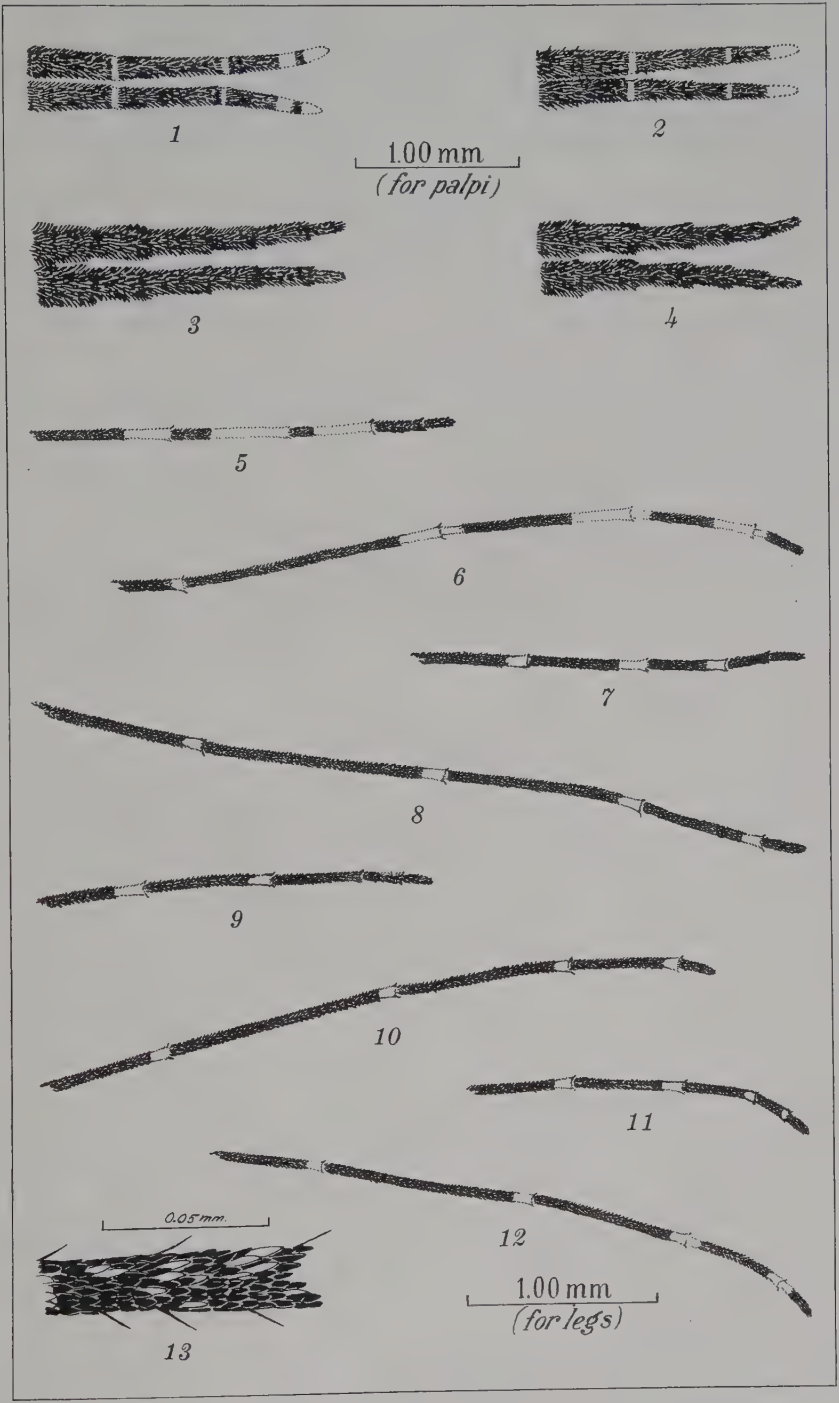
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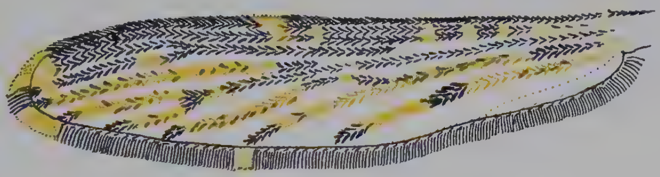
PLATE 1.



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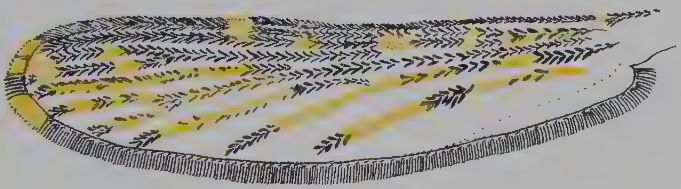

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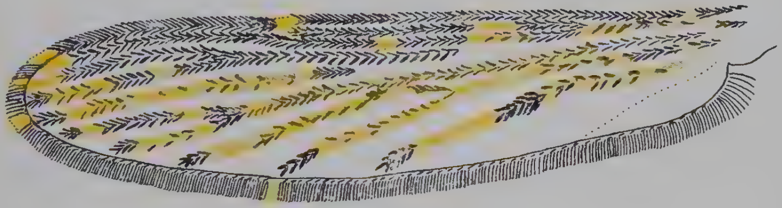

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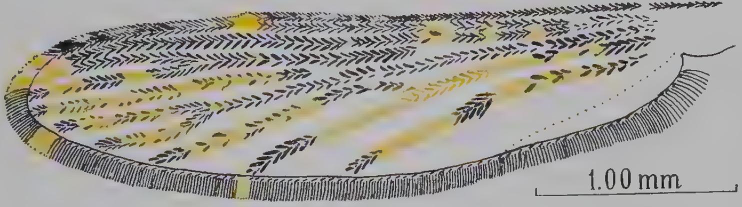

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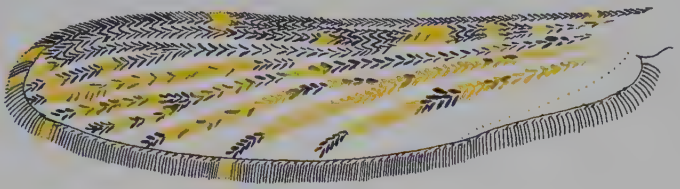
PLATE 2.



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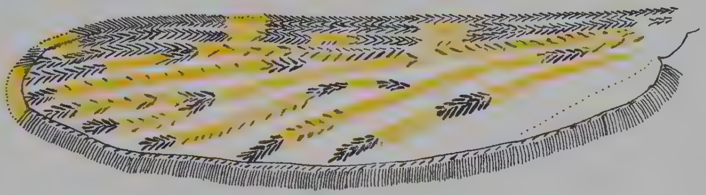

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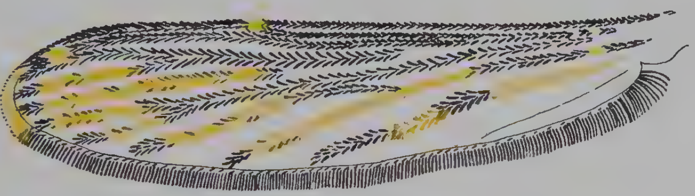

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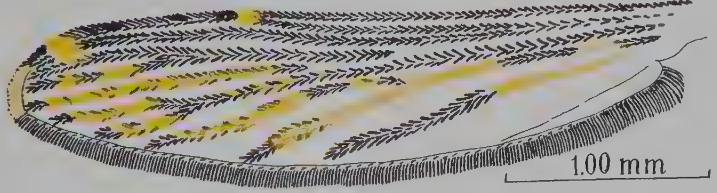

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PLATE 3

REPORT OF THE LEONARD WOOD MEMORIAL CONFERENCE ON LEPROSY

HELD IN MANILA, PHILIPPINE ISLANDS, JANUARY 9 TO 23, 1931

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FOREWORD

It has long been evident to those engaged in the study and control of leprosy, that the existing terminology and classification of the subject permitted of widely divergent interpretations. This defect had become a handicap of such degree in correlating the views and results of different leprologists, that an opportunity for discussion and attempted correction of some of the difficulties seemed to be an imperative requirement for progress.

During the past year both the Leonard Wood Memorial for the Eradication of Leprosy and the Leprosy Commission of the League of Nations took cognizance of the situation, and became interested in holding a round-table conference, in the Far East, and the trustees of the Memorial subsequently made a financial allotment to effect this purpose in so far as it was practicable. It was decided that the Conference should take place in Manila immediately after the meeting of the Leprosy Commission, which was held in Bangkok in December, 1930.

The number of leprologists invited to the Conference was necessarily limited since it was intended that the proceedings should be of the nature of informal discussions. It seemed especially desirable that those individuals who are members of the Leprosy Commission of the League of Nations, or their representatives, should attend because of their previous studies of the subject, and of their broad knowledge of the conditions prevailing in the widely scattered areas which are affected. Invitations were extended to a number of others who were located in the Far East, and whose experience was known to include unique aspects.

The Conference convened at Manila January 9, 1931, and was in session until January 23, 1931. Those who attended are as follows:

Dr. Et. Burnet, secretary, Leprosy Commission, League of Nations.
Dr. R. G. Cochrane, secretary, British Empire Leprosy Relief Association.
Dr. H. I. Cole, chief chemist, Culion Leper Colony.

Dr. J. FAJARDO, director of health, Philippine Health Service.

Maj.-Gen. J. D. GRAHAM, I. M. S., public health commissioner, Government of India.

Dr. G. GUSHUE-TAYLOR, superintendent, Mackay Memorial Hospital, Taihoku, Formosa.

Dr. V. G. HEISER, director for the Far East, Rockefeller Foundation.

Dr. LEE S. HUIZENGA, superintendent, Mission Hospital, Jukao, Ku, China.

Dr. A. N. KINGSBURY, director, Research Institute, Kuala Lumpur, Federated Malay States.

Capt. P. H. J. LAMPE, director of health, Dutch Guiana.

Dr. C. B. LARA, chief physician, Culion Leper Colony.

Dr. J. LOWE, medical superintendent, Dichpalli Leprosy Hospital, India.

Dr. J. L. MAXWELL, Henry Lester Institute of Medical Research, Shanghai.

Dr. E. MUIR, leprosy research worker, Calcutta School of Tropical Medicine.

Dr. E. E. NEFF, superintendent, Mogokai Central Leper Hospital, Fiji.

Prof. Dr. B. NOCHT, president, Leprosy Commission, League of Nations.

Dr. M. OTA, Tohoku Imperial University, Japan.

Dr. J. N. RODRIGUEZ, supervisor of leprosy-treatment stations, Philippine Health Service.

Dr. J. C. TULL, government pathologist, Singapore.

Dr. H. W. WADE, chief pathologist, Culion Leper Colony; and medical director, Leonard Wood Memorial.

Dr. N. E. WAYSON, director, United States Leprosy Investigation Station, Hawaii.

Drs. LEROY-DESBARRES and H. JOYEOUX, of French Indo-China, also participated in the first sessions.

The Conference was organized at the first meeting, and the agenda for consideration was agreed upon promptly thereafter. The subject matter was prepared for the most part in committee, presented to the plenary sessions, and final decisions reached in these. January 14, the members embarked on a journey of inspection of regional treatment stations and leprosaria at Cebu, Zamboanga, and Iloilo, and of the Culion Leper Colony. The sessions were continued with but brief intermissions throughout the tour, both on the steamer and at ports visited.

The discussions were frank and detailed, and were in most instances extended to a point of reconciliation of viewpoints and common agreement. The following report is that decided upon by the Conference in plenary sessions.

H. W. WADE, G. GUSHUE-TAYLOR,
Chairman of Conference. *Secretary.*

DISCUSSIONS AND CONCLUSIONS

I. THE INTERNATIONAL VIEWPOINT

An important cause of misunderstanding among leprosy workers is their insufficient appreciation of fundamental differences in conditions and practices existing in different regions. Leprosy has a wide range of manifestations and the predominating features, such as the frequency of one or another type, may vary widely in different regions. Local conditions of various kinds must determine the nature of antileprosy activities. The results of work may be influenced by such factors as the type of case, the degree of advancement of the disease, and the conditions under which the patient is observed. It is important that leprologists bear such factors in mind in order that they may precisely evaluate their own findings, particularly with regard to the work of others, and avoid the formulation of unduly broad conclusions.

Study tours.—It is deemed highly advisable that any country or institution undertaking serious work in leprosy should send some of its leading workers to one or more of the main centers of leprosy activities, in order to study the local conditions and the methods in use. The value of this lies not only in the knowledge acquired that can be applied directly to the visitor's own work, but also in the broadening of his outlook on the leprosy problem as a whole.

Transfer of workers.—It not infrequently happens that the results obtained by a worker or group in one country are not confirmed by those working in other countries. Whether this is due to peculiarities of conditions prevailing, or to the personal equation, or to other factors is usually not apparent. Progress toward the clarification of questions of regional differences could undoubtedly be accelerated were it possible for persons who have carried out studies in one region to be transferred to another in order to continue or repeat such studies there or to undertake correlative investigations. It is deemed desirable to bring the possibilities of such a plan to the notice of institutions and organizations concerned with the study of leprosy.

Leprosy digest, or annuaire.—It is unquestionably difficult for isolated workers to gain from the available literature an adequate understanding of conditions prevailing in other regions. There is need of a reference work of the nature of a comprehensive leprosy survey, or annuaire, which would afford a reasonably accurate summary of what is known of the leprosy situation in all parts of the world. It should include information regarding the extent and distribution of the disease in the various countries or regions, the predominating types, other factors that affect leprosy work, and the laws and regulations obtaining; it should also include statements of activities, lists and brief descriptions of institutions, and perhaps data concerning active leprologists. The existing information of this nature is widely scattered and incomplete. It should be collected, summarized, amplified where necessary, and brought up-to-date. The preparation and periodical revision of such a survey would be a laborious and somewhat expensive task, hardly possible except through the agency of an organization such as the Leprosy Commission of the League of Nations or an association of leprologists. The matter should, however, be given serious consideration.

II. UNIFORMITY OF METHODS AND TERMS

Differences in terminology, in classification and subclassification of cases, in methods of examination and treatment, and in other features are felt to be a serious handicap to leprosy work in general. There is little possibility of arriving at uniformity of practice in certain features, notably the methods used in the general antileprosy campaign, but in other matters it should be feasible to arrive at some degree of standardization.

1. DESIGNATIONS OF CASES OF LEPROSY

It is confusing, when there is need of precision, if the term "case of leprosy" is applied indiscriminately to all types, from those that are active but with slight involvement, to those with marked mutilations but in which all evidence of activity has long since disappeared and only the permanent sequelæ remain. Reports should be precise, indicating the types of cases, and the subtypes based on degree of advancement (see page 457); the proportions of each should also be stated. Unless this is done it is impossible to compare the results obtained in different institutions. There should, also, be uniformity in the use of other terms employed in designating cases of leprosy.

The term "suspected" case applies to a patient who presents clinical signs that are suggestive of leprosy but not sufficient for a definite diagnosis.

The term "clinical" case is sometimes applied to distinguish one that is diagnosed on clinical grounds, the bacteriological findings being negative.

The term "incipient" case is sometimes applied to an early or slight case in which the bacteriological findings are negative; this has been used where the "cutaneous" type greatly predominates and patients with bacteriologically positive findings are isolated.

Since the terms "clinical" and "incipient" are to a certain extent indefinite, it is preferable that whenever possible more specific terms be used (see page 455).

The term "infectious" case is much used, chiefly in connection with administrative measures. The use of this term is not recommended; attention is called to Administrative Classification of Cases, page 458.

The term "burned-out" case is often applied to arrested cases that show mutilations, contractures, etc. It is recommended that the use of this term be discontinued and that "arrested with deformity" be employed.

The term "leper" is felt to carry with it a definite social stigma. It is recommended that the use of this term be discontinued and that "case of leprosy" be employed.

2. DESIGNATION OF LESIONS

Leprosy may produce pathological changes in many tissues of the body. The nerves, skin and mucous membranes, eyes, bones, lymph glands, testes, and other organs may be affected. For present purposes, attention will be confined to those changes which most commonly produce characteristic clinical manifestations.

Lesions of peripheral nerves may produce thickening, local tenderness, and, rarely, abscess formation. The signs associated with nerve involvement tend to fall into two groups, namely: (1) The *macular*, which is characterized by disturbances of pigmentation, circulation, and sensation in circumscribed areas. (2) The *acroteric*, in which the changes occur in the extremities with a tendency to centripetal spread. These include alterations of sensation; trophic disturbances in the skin, bones, and muscles; and paralyses. The signs of both groups may coexist and either may predominate.

Changes in the skin may be functional, structural, and circulatory. Functional changes are hyperesthesia, hypoesthesia, paresthesia, anæsthesia, hyperhydrosis, anhydrosis. Structural changes are keratosis, hypopigmentation, hyperpigmentation, depilation, atrophy (parchment skin or crushed tissue-paper appearance), infiltration (thickening), suppuration, ulceration (leprotic, trophic, traumatic), scarring. Circulatory changes are hyperemia, ischemia, œdema (circumscribed or diffused), bullæ.

DEFINITIONS

Leprotic.—It is suggested that the term "leprotic" be applied to those changes which present clinical or microscopic evidence of inflammatory processes, typically of granulomatous nature, which are apparently caused by *Mycobacterium lepræ* in them. In such lesions the organism can usually be demonstrated by the ordinary methods of examination.

Leproma.—The term "leproma" is applied in a general sense to any lesion of a leprotic nature, as defined herein.

Ulcers.—In considering the ulcers of leprosy, distinction is not always made between those which occur in leprotic lesions and from which bacilli are usually discharged, and those which are sequelæ of nerve changes. The former type, occurring in leprotic tissues, should be called "leprotic ulcers." The latter, which usually occur in nonleprotic tissues and do not discharge bacilli, should be called "trophic ulcers." Traumatic ulcers are particularly liable to occur in tissues affected by trophic changes.

Infiltration.—"Infiltration" is a term commonly applied to a diffuse thickening of leprotic nature involving the skin or mucosa which is not of definite nodular, papular, or macular form. The term may also be applied to diffuse leprotic conditions in other organs.

Nodule.—A nodule is a definitely thickened, rounded, circumscribed mass of leprotic nature commonly occurring in the skin, subcutaneous tissue, or mucosa.

Papule.—A papule is a small solid elevation of the skin, of leprotic nature, not more than 5 millimeters in diameter.

Macule.—A macule is a circumscribed area of skin showing changes in color, sometimes with slight elevation or depression. The following descriptive terms may be applied to indicate its peculiar characteristics: Hypopigmented, hyperpigmented, erythematous, circinate, marginate, zonal, raised, atrophic.

3. THE MICROÖRGANISM

Nomenclature.—There is considerable variance in practice in the formal designation of the organism of leprosy. Common practice countenances the informal use of the term "bacillus" for rod-shaped organisms in general. The generic name *Mycobacterium* is now extensively used for the acid-fast group to which the organism of leprosy belongs. Therefore, while it is permissible to speak colloquially of "the leprosy bacillus," it is recommended that it be not referred to formally as *Bacillus lepræ* (*B. lepræ*), but as *Mycobacterium lepræ* (*M. lepræ*).

"Toxins."—Attention may be drawn to the fact that "toxins" of *M. lepræ,* in the sense used in bacteriology, are hypothetical. It is a striking fact that a patient with extensive and progressive cutaneous lesions containing incalculable numbers of microorganisms may show no clinical indication of any kind of toxic effect. In the condition known as "lepra reaction" toxic manifestations are seen, but there is reason to believe that these are not due to true bacterial toxins. In the present state of our knowledge it is advisable to avoid the use of this term.

4. CLINICAL CLASSIFICATION OF CASES

The classification of cases of leprosy is an important but difficult matter. Even with the same viewpoint no two workers in leprosy may necessarily classify any large group of patients precisely alike, because of differences of interpretation and of skill and care in examination. Thus, the confusion arising from the present differences of viewpoint and practice makes it imperative that a serious attempt be made to arrive at some uniformity in this matter.

Primary classification; types.—Leprosy is a general disease; in no type are the lesions confined to a single tissue, and the demonstration of bacilli is not always possible by routine methods. All diagnosable cases are in a sense "mixed," and in consequence any classification should be based primarily on the predominating clinical findings.

This Conference is in agreement that cases of leprosy should be divided into two major types, and that these be designated "neural" and "cutaneous." The division is based on the following considerations.

From clinical and pathological evidence it seems clear that the leprosy bacillus has a very special affinity for peripheral nerves. Clinically there are many cases that, for practical purposes, may be considered essentially neural. They frequently have visible (usually hypopigmented) lesions of the skin, but in typical cases the bacteriological findings by the usual methods are consistently negative. However, it is the experience of some observers that in occasional typical cases bacilli may be found in the nasal mucosa, though in their clinical course neural cases differ markedly from those with "leprotic" skin lesions (see page 453). Therefore, regardless of the manner in which the bacillus has entered the body, the mechanism of the production of the skin disturbance, or the possible presence of the bacilli in other tissues such as the lymph nodes, it may be agreed that it is reasonable and useful to recognize a nerve, or "neural," type of leprosy. It is to be realized that neural cases may become "cutaneous" through the subsequent development of leprotic lesions of the superficial tissues.

On the other hand, pure leprosy of the skin does not exist as a type. It may be that in exceptional cases lesions of the skin, in which *M. lepræ* is demonstrable, may exist before lesions develop elsewhere; but there is reason to believe that in such cases the bacillus almost invariably becomes disseminated and causes lesions in other organs, especially the nerves. Furthermore, in many cases that ordinarily would be classed as "cutaneous" leprosy, a history of primary nerve disturbance can be obtained, and very often careful examination may reveal some degree of sensory disturbance and of nerve thickening. Therefore, on this evidence, such cases are strictly "mixed." Sooner or later, in the natural course of the disease, gross evidence of neural involvement (atrophies, mutilations, paralyses) may be expected to develop. Notwithstanding these facts, in order to minimize confusion, it is deemed desirable to class all cases with leprotic lesions of the skin as "cutaneous."

Cases that have once been cutaneous, but with important neural manifestations (that is, the old "mixed"), and have improved until only the neural lesions or sequelæ remain, require separate recognition. If first seen in this condition they would ordinarily be classed as advanced neural, but they should be considered "secondary neural" in contradistinction to the "primary neural" cases, which have never apparently been of the cutaneous type.

PROPOSED CLASSIFICATION OF TYPES OF LEPROSY

A. MAIN TYPES

Neural (*N*).—All cases that show evidence of actual or previous nerve involvement; i. e., alterations of sensation with or without changes in pigmentation and circulation, trophic disturbances or paralyses and their consequent results: atrophies, contractures, ulcerations. These are not accompanied by leprotic changes in the skin.

Cutaneous (*C*).—All cases showing leprotic lesions in the skin. Such cases may or may not show, at any given time, clinical manifestations of nerve involvement.

B. SUBTYPES (INDICATING DEGREE OF SEVERITY)

Neural–1 (N–1).—Slight neural: Cases with one or a few small areas of disturbed sensation, which may or may not show alterations of circulation or pigmentation, paralyses or trophic disturbances of minor degree.

Neural–2 (N–2).—Moderately advanced neural: Cases with extensive or numerous areas of disturbed sensation, not confined to any one part of the body; with paralyses or/and visible evidences of trophic disturbances: marked depigmentation, moderate atrophy, keratosis, bullæ, etc.

Neural–3 (N–3).—Advanced neural: Cases with more or less extensive areas of anæsthesia and marked motor and trophic disturbances; marked paralyses, atrophies, contractures, trophic ulcers, and mutilations.

Cutaneous–1 (C–1).—Slight cutaneous: Cases with one to a few leprotic macules, or a few small areas of infiltration, or nodules.

Cutaneus–2 (C–2).—Moderately advanced cutaneous: Cases with numerous leprotic macules, or fairly numerous or marked areas of infiltration, or nodules, frequently with lesions of the mucosa.

Cutaneous–3 (C–3).—Advanced cutaneous: Numerous or very marked leprotic lesions in various stages of development or retrogression, usually with lesions in the mucosa.

In all cutaneous types there may be varying degrees of neural involvement and such cases should be recorded to indicate the degree of this involvement; as, for example, C–2, N–1.

Secondary neural.—Neural cases that were formerly cutaneous, but from which the active leprotic lesions have disappeared.

5. ADMINISTRATIVE CLASSIFICATION OF CASES

In order to facilitate the application of preventive measures to leprosy, it is desirable to reduce probable sources of infection. To accomplish this it is necessary to recognize that leprosy is probably perpetuated through the implantation of the organism in favorable environment, such as new susceptible hosts. This implantation is believed to be effected through the dissemination of *M. lepræ* from the mucous membrane, skin, and perhaps from the excretions of the infected host. We have no absolute criteria of infectivity, but experience has shown that in cases in which the organism can be found in the skin or mucous membranes it may also be found at times on the surfaces of these.

Such conditions would thus favor its dissemination. Cases from which dissemination seems probable should therefore be classed as "open;" while other cases may be considered "closed." Administrative action concerning the two classes might well differ in its application according to the community affected.

Closed cases were formerly described as "bacteriologically negative;" but since this implied that no bacilli could be obtained from the accessible lesions by the ordinary methods of examination and since the expression might be misinterpreted to mean that there were no bacilli in the tissues, it is recommended that it be replaced by the term "closed" in administrative practice.

6. LEPRA REACTION

Lepra reaction is one of the most interesting and important phases of the disease. It is of frequent occurrence and may vary from a painful neuritis or the reddening of an isolated skin lesion to a general exanthematous eruption or a sudden marked increase in the severity of existing lesions with ulcerations and toxic manifestations, and if protracted may lead to death. Such reactions are frequently referred to as "acute exacerbations" of the disease. This may be misleading. At times lepra reaction may, indeed, initiate an actual advancement of the disease and on subsidence of the acute reaction the original lesions may be larger or more numerous than before. On the other hand lepra reaction may prove beneficial, for after its subsidence the lesions may improve.

This phenomenon and its therapeutic control should be subjected to further study. The whole subject of the value of artificially induced reactions in therapeusis also needs further investigation (see Appendix III).

7. EARLY DIAGNOSIS OF LEPROSY

Special stress is to be laid on the importance of the diagnosis of early and slight cases of leprosy, and of their treatment, both from the point of view of prevention of the disease and of relief of the patient. The sooner a patient is put under treatment the more promising are the results and the less the danger that such a patient will infect others, if the prevailing view that the disease is transmitted from man to man be correct. This view the Conference upholds.

All persons responsible for the diagnosis of leprosy should, therefore, acquaint themselves with the signs and symptoms diagnostic of leprosy in its earliest stages (see Classification of Cases, N–1 and C–1).

8. METHODS OF EXAMINATION

Accuracy of diagnosis and the evaluation of improvement depend upon the methods of clinical and bacteriological examination employed and upon the care and skill with which they are applied.

Clinical.—The use of crude methods as well as careless practice may result in failure to detect cases of slight degree and may also lead to gross errors in estimating the progress of the case. The importance of adopting proper methods of clinical examination cannot be overemphasized (see Appendix I).

Bacteriological.—The significance of the bacteriological findings cannot be considered other than more or less arbitrary. In routine practice the examination is confined to limited portions of the superficial tissues, skin, and nasal mucosa, and even repeated negative findings do not prove that *M. lepræ* is absent therefrom. It can usually be assumed that in neural cases the organisms are present in the nerves at least and that in cutaneous cases they are present also in the deeper organs (see Appendix I).

9. TREATMENT OF LEPROSY

The treatment of leprosy that seems most likely to succeed includes both general and special measures. These measures should be adjusted to the nature of the individual case, and not applied indiscriminately.

General measures.—The general measures that appear to be most applicable do not differ materially from those used in other infectious diseases whose course is often of great chronicity. However, it is believed by physicians experienced in this disease that careful and persistent efforts to eliminate intercurrent affections which tend to reduce the general resistance of the patient are essential to successful therapy. Observations suggest that the adjustment of both the quality and the quantity of the diet may be an important therapeutic measure. It is also the belief of those with experience that other general measures, including personal hygiene, supervised or graduated physical exercise, occupational therapy, the stimulation of morale, and mental welfare are also of definite value.

Special measures.—Special measures include those that may stimulate the general healing processes, and those that attempt to effect the resolution of individual lesions. Such measures include the administration of drugs and the application of physical agents.

The drugs most widely used are oils or their derivatives. The oils in very common use are those of *Hydnocarpus wightiana* and *H. anthelmintica* (chaulmoogra group). The derivatives of these oils that are receiving most attention among clinicians treating leprosy are the ethyl esters of the respective fatty acids and the soaps (sodium salts) of these acids. Available clinical evidence indicates that the therapeutic value of these oils is similar, and this is true also of their derivatives. The choice of these for use in treating leprosy may therefore rest on other factors; such as, the ease with which they may be procured, their cost on delivery, their purity and freshness, and their keeping qualities.

The ethyl esters have been intensively used in large treatment centers and are preferred by the workers in those centers, as well as by others. The preference for this derivative seems to be influenced by the fact that a process of its manufacture has been attained that permits of the repeated production of a stable, uniform, and standardized product; also by the fact that when administered by injection, it produces less local irritation than that caused by some other derivatives. The degree of irritation produced by the injection of the esters may be reduced by the addition of metallic iodine (0.5 per cent) to them (see Appendix IV). However, other workers have preferred to use either the oils, or the sodium salts of their fatty acids. These drugs, also, can be administered by injection, intramuscularly or subcutaneously. They have not been used sufficiently by intradermal injection to admit of comparison with the esters.

The methods of injection may be intramuscular, subcutaneous, intracutaneous, or intravenous. Selection will be influenced to some degree by the nature of the medicament used; for example, it is difficult to inject the oil intradermally, and the esters or a solution of the soap is preferable when this method is used. Attention is called to the fact that oils and esters should not be given intravenously. Recent evidence tends to show that lesions of the skin resolve more rapidly when treated by intradermal injections. The dosage of these various preparations cannot be arbitrarily established, but must be adjusted to the condition of the individual.

Other oils have been used in a manner similar to that adopted with the hydnocarpus group, but experience of their use has not been sufficient to permit of an appraisal of their value.

To stimulate reparative processes in a patient measures have been adopted for the production of a controllable febrile reaction, not of a specific nature. These measures have included the use of preparations such as foreign proteids, either of bacterial or of other origin, or exposure to physical agents. The physical agents employed for this and for special purposes in the treatment of leprosy include ultra-violet light, radiant heat, hot baths, carbon dioxide snow, fulguration, and diathermy.

Other drugs, especially preparations of gold and other heavy metals, have been advocated for use in special cases, but such drugs should be employed with caution. Iodides have been employed, but they are contraindicated in many cases. Experience has shown that their use should be limited to patients whose general condition is such that they can tolerate full doses of hydnocarpus preparations and maintain at the same time good general health. Adequate control by those skilled in the treatment of the disease is essential.

Standardization and appraisal of treatment.—It is impracticable at this time to establish a standard method of treatment. However, a method that has proved satisfactory is the intradermal injection of preparations of the hydnocarpus (chaulmoogra) group of oils, preferably combined with intramuscular injections (see Appendix II).

It must be recognized that the course of leprosy is often of great chronicity, and final conclusions concerning the therapeutic value of a drug or method cannot be reached until after its use for one to several years. Further, it must be appreciated that there is no accurate method of measuring such therapeutic value. Both clinical estimates and microscopic examinations are subject to many errors. Skill, care, and adequate staff are necessary to carry out these examinations accurately.

Organization of treatment.—This will vary with the number and type of cases to be treated, and with the available personnel and facilities. It will also depend upon the political, geographical, and economic conditions of the community, as well as upon the attitude of the patients and of the general public towards the whole subject. For purposes of treatment, cases may be grouped into classes in accordance with their probable response to treatment and their prognosis regarding arrest or recovery. Under favorable conditions, such classes may be cared for in separate sections of an institution or, preferably, in separate institutions. While certain types and classes of patients may be adequately treated as out-patients, it will be apparent that cases requiring close supervision and those who are incapacitated should have institutional care. Under the latter conditions clinical observations can be made more accurately and the patients' regimen can be controlled.

10. EVALUATION OF PROGRESS

In the evaluation of progress it is necessary to use terms to define the stage to which the patient has progressed. Such terms are indicated below. Cases may be classified with regard to the course of the disease as active, quiescent, and arrested.

Active cases.—"Active" cases are those in which there are clinical or microscopic evidences of progressive or of recessive changes in lesions, with or without accompanying systemic disturbances.

These evidences include the following: Positive bacteriological findings in skin or mucous membrane determined by the usual methods; the presence of raised or erythematous lesions; increase or diminution of lesions in size or number; tenderness of nerves, with or without thickening.

Quiescent cases.—"Quiescent" cases are those in which there is no longer clinical or microscopic evidence of activity as defined above.

For the purpose of attaining uniformity of method, the classification "quiescent" should be applied to cases from which the signs of activity have been absent for a period of at least three months. This absence of activity should be determined by at least one examination a month during this period. This examination should include a clinical examination, and also microscopic examinations of the nasal mucosa at more than one site, and of lesions of the skin at more than two sites.

Arrested cases.—"Arrested" cases are those that have remained quiescent for a period of at least two years.

Cure; relative cure; negative.—Use of the term "cure" in relation to the results of the treatment of leprosy is open to misunderstanding; and it is, therefore, recommended that this term should not be employed in the terminology of leprosy. Since the term "negative" is often misinterpreted and the term "quiescent" is more descriptive of progress than "negative," it is recommended that the term "negative" as applied to patients likewise be discontinued. It is further recommended that the term "relatively cured" be not used, since it includes the word "cure;" and that the condition to which it is applied be described by the term "arrested."

11. FOLLOW-UP OF PATIENTS

This Conference considers it desirable to lay emphasis on the need of following up, over a considerable period of years, patients who have apparently recovered under treatment, in order to ascertain the ultimate results thereof. This is important from the scientific and public-health viewpoints, as well as from that of the welfare of the patients. The findings of certain workers who have made a survey of released cases open to question the permanency of the results obtained in recent years. Further knowledge of this matter is greatly needed.

12. STUDY OF CONTACTS

The opinion expressed at the last international conference on leprosy was to the effect that leprosy is a communicable, rather than a congenitally acquired, disease. No satisfactory evidence has been adduced that would invalidate this opinion. Therefore, it is desirable to emphasize the importance of studying the contacts of known and especially of "open" cases. Such work may be difficult and expensive, but the studies should throw important light on the epidemiology of the disease and on its earliest manifestations. It may be that the eradication of leprosy from any given area will depend upon the application of knowledge so gained.

13. STANDARDIZATION OF RECORDS

The Conference strongly indorses the conclusion of the Leprosy Commission of the League of Nations concerning the desirability of attempting to standardize the forms used in recording and reporting leprosy work. It feels that this is of great importance in the comparison of results obtained by different observers, which is almost impossible at the present time.

As the work will entail the collection and detailed study of forms now in use in various active institutions throughout the world and can best be done under the auspices of a permanent organization, the Conference resolves to invite the Leprosy Committee of the League of Nations to undertake this and, after preparing standardized forms, to circulate these to leprologists throughout the world.

III. PROPHYLAXIS

The Conference has not specifically dealt with the subject of prophylaxis in leprosy.

The findings of the Bangkok meeting (1930) of the Leprosy Commission of the League of Nations, which have not yet been released for publication, were placed before it.

The Conference gives its approval to these findings in principle.

IV. RESEARCH

While there are many facts regarding leprosy that may not be known even after intensive and prolonged research, there are others that require prompt investigation because of their immediate importance in more effective control of the disease. A recent survey made by the Leprosy Commission of the League of Nations has also shown that there is lack of coördination in the research now in progress. The following program of subjects, which seem to be in urgent need of investigation, is therefore submitted.

1. EPIDEMIOLOGY

It is believed that studies in the epidemiology of leprosy are greatly needed. There are serious defects in our knowledge of the incidence with regard to race, geography, environment, occupation, climate, family history, age, sex, and diet; also the incubation, duration, apparent spontaneous disappearance, incidence among healthy attendants in leprosaria, spread in newly invaded areas, apparent immunity in certain districts or areas as compared with others, etc.

Comparison of observations made over widely scattered areas is highly desirable. In order that the data collected in such observations may be of the greatest value, they should be as complete as possible, systematic, and made by units of trained personnel. An outline that includes most of the details considered essential to accomplish this is appended (see Appendix V).

However, much can probably be learned from surveys that cannot be made in so intensive a manner, and it is suggested that these should include as many of the outlined data as may be feasible.

Any survey might well include inquiries into native folklore, traditions, and superstitions regarding the disease.

2. CLINICAL STUDIES AND THERAPEUTIC EXPERIMENTATION

The clinical course of the disease has been studied, for the most part, in cases that are now classified as of moderate or marked advancement. It is of great importance that studies be made for the purpose of detecting the disease in its earliest stages. The clinical observations in such cases should be supplemented by the development of technical procedures which may be applied to the patient or executed in the laboratory. Investigations should be made of individuals who may not have shown clinical evidence of the disease and of those who have apparently recovered. Such investigations might be regarded as those of latent leprosy, and may include various cutaneous tests or cuti-reactions with specific vaccines, and the determination of the persistence of *M. lepræ* in the nasal mucosa.

The clinical phenomenon known as "lepra reaction" is worthy of particular attention, because of its frequent occurrence in the course, and because of its apparent alteration of the course of the disease. Its causes, nature, and control are still undetermined.

Investigations of clinical phenomena should be intimately correlated with the effect of therapeutic measures on the patient. This is especially pertinent, because of the failure to produce in animals conditions that closely resemble human leprosy. The interpretation of pharmacological investigations in animals should be considered with regard to their probable effect in man. Some remedies may produce marked disturbances in the course of the disease, and their use may be dangerous in certain stages, while in others they may be beneficial. The use of iodides, especially potassium iodide, should be studied in this connection. Their employment, however, should be attended with extreme caution; and it has been suggested that the indication for their administration may be determined and controlled by the application of the red-blood-cell sedimentation tests. Various remedies for certain phases of the disease should be explored, such are exemplified by the use of tetradotoxin and dilo oil (*Calophyllum bingator*) in lepra reaction. There is also further need for carefully controlled tests and more accurate appraisal of the special remedies now commonly used, and for those of physical therapy; such as, electrotherapy, mechanotherapy, hydrotherapy, actinotherapy, and physiological exercise.

3. DIET

Differences have been noted in the incidence of leprosy in the people of various regions in India, Korea, China, and other countries, which suggest that intensive studies of the diet of the people are desirable. These studies should include the foods in use, the methods of preparation, the degree of preservation (freshness, decomposition), the quantity (famines), and the quality (vitamins, inorganic salts). The effect of diet in general therapy is also of importance.

4. ETIOLOGY AND PATHOGENESIS

The entire subject of the pathogenesis of leprosy is in need of investigation, and this should comprise studies of *M. lepræ,* including the possibility of its growth in cultures of tissue; its chemical and biological relationship to other bacteria of the acid-fast staining group; and its behavior in animals when inoculated from suspensions made from lesions, but freed of tissue. Efforts should be continued towards finding an animal susceptible to inoculation (anthropoid apes); the preferable site of inoculation; the effect of massive doses of the inoculum; and repeated inoculations. Animal diseases that resemble leprosy (rat leprosy) may also afford a fruitful field.

Research is urgently needed concerning the site and manner in which the organism invades the body; its dissemination through the tissues and its seeming selectivity for certain tissues and sites in them; and the pathological changes produced by it in the first or early clinical lesions, in leprides, in the nerves throughout their course, and in the bones and blood vessels as well as in the skin. The pathology of the tissues of the patient who has been treated should be investigated.

These studies should also include those of immunity, and should embrace serological and cytological studies (lepra cells), allergy, and the relation of vaccines of *M. lepræ* and other acid-fast organisms. (Tubercle bacillus B.C.G.)

5. BIOCHEMISTRY

Experience with leprosy suggests that the metabolism of the case of leprosy is deranged, and it is also thought that the type of the food consumed by the patient may alter his suceptibility to the disease. It is evident, therefore, that biochemical studies are needed in this regard in metabolism, vitamins, inorganic salts, proteids, carbohydrates, and fats. Knowledge of chemistry of the blood (lipoidal content) may also be of great value in interpreting various phases of the course of the disease.

6. PHARMACOLOGY

The pharmacological action of remedies (for example, metals) applied to this disease may be different from that in others; and it is, therefore, necessary to investigate the special action of drugs that have been used and to develop others, which may be of greater value. Such studies may well be made of the action of the drugs now in use on *M. lepræ*, the action of the various preparations of the hydnocarpus group (and of other oils), native remedies, synthetic remedies, new remedies, the selective action of drugs, and the relation of the secretions of the endocrine glands.

APPENDICES

I. DETAILS OF EXAMINATION

(*a*) *Clinical examination.*—The whole body should be examined, in so far as is possible, in a good light and all the findings accurately recorded. While many auxiliary tests may be employed, the following methods are important:

1. Sensation to light touch.—The patient should be blindfolded. The normal skin should be repeatedly touched with some light object, such as a cotton swab, a feather, a camel's-hair brush, or a spill of paper, and the patient asked to indicate accurately with the point of the finger the place touched. When the patient is responding to these stimuli, the suspected skin areas should then be similarly tested, loss of sensation to light touch being indicated by repeated failure to respond. In this way anæsthetic areas may be gradually mapped out.

2. Sensation to pain.—The eyes being blindfolded, a suspected skin area is alternately touched with the head of a pin and pricked with the point, a corresponding normal area being similarly examined immediately afterwards. The patient is questioned as to which prick produces more pain, the touch with the head or the prick with the point. This process should be repeated several times so as to avoid error.

3. Sensation to heat and cold.—This may conveniently be tested under similar conditions by touching suspected areas with two test tubes alternately, the one containing hot (40 to 50° C.)

and the other cold water (20° C. or lower), the patient being asked to distinguish between them.

4. Thickening of the skin.—The detection of slight degrees of skin thickening often requires considerable care. Inspection, with or without a magnifying glass, should be supplemented by palpation, the suspected area being rolled between the finger and thumb. Comparison should be made with the surrounding skin and with the corresponding area on the other side of the body.

5. Thickening and/or tenderness of the nerves.—The superficial nerve trunks in normal individuals are frequently palpable and firm pressure may elicit slight pain. The determination of thickening and abnormal tenderness should depend on careful comparison with the nerve, if unaffected, on the other side of the body or with the corresponding nerve in a healthy person of similar build. The superficial nerve trunks most commonly affected are the ulnar, the superficial peroneal, and the great auricular. Sensory branches supplying macules are sometimes tender and palpably thickened.

6. Mucous membranes.—In examining the nasal mucosa it is advisable to use a speculum, and the field should be well illuminated.

(*b*) *Bacteriological examination.*—Particular stress is to be laid on the need of examining smears from several sites and of making repeated examinations. Organisms may be demonstrable in one lesion or in only one part of it, while in another lesion they cannot be detected. In the early progressing cutaneous case the organisms in a lesion may be few and scattered, later becoming more numerous and generalized, while the converse may be true in the case as it improves.

Smears should contain as little diluting material (blood, lymph) as possible, but the specimen should be so taken as to contain cellular material from the deeper layer in which the organisms are normally to be found.

1. Skin examination.—There are two principal methods of procuring material for examination; namely, the "scraping" and the "snip." By the former a very small cut, about 2 millimeters deep, is made with the scalpel well into the dermis, and material is scraped from the depth of this and smeared on a slide. By the latter method a small portion of the dermis, at least 2 millimeters thick, is snipped off with a sharp pair of scissors, curved on the flat. The raw surface of the tissue so obtained is applied to a slide and firm pressure is exerted so as to express as much as possible of the cellular elements.

2. Nasal examination.—With the use of a nasal speculum the interior of both nares is carefully examined for infiltrations, nodules, and ulcers. If any of these is found, material should be removed therefrom with a blunt narrow-bladed scalpel, or a similar instrument, by scraping deep enough to cause slight bleeding. Even when there is no visible lesion, a scraping should be taken from the septum. *Mycobacterium lepræ* may be found on the septum, the inferior and middle turbinates, or the floor of the nose. The material so obtained should be smeared on a slide.

3. Staining.—After drying, and fixing over a flame, smears are stained for at least ten minutes at room temperature or heated three minutes till steam rises, in a solution of carbol fuchsin. This is prepared by mixing one part of a 10 per cent solution of basic fuchsin in 90 per cent alcohol with nine parts of a 5 per cent solution of carbolic acid crystals in distilled water. This solution should be prepared at frequent intervals and be discarded when there is any trace of precipitate.

The slide is decolorized with sulphuric acid (10 per cent) or nitric acid (10 to 20 per cent) in water, and counterstained with methylene blue.

II. METHOD OF TREATMENT BY INTRADERMAL INJECTION

A scheme of treatment in use at some of the largest centers is as follows:

The ethyl esters of hydnocarpus oil, which are prepared in accordance with the methods outlined on page 471, are preferred, but a stable uniform preparation of the sodium soaps of the fatty acids of the oils of this group may be used.

The injections may be given at weekly intervals. Not more than 5 cubic centimeters are given to a patient at any one time, and not more than 0.1 cubic centimeter is injected at any one point in a lesion. Whenever feasible, the lesion to be injected is completely infiltrated by producing coalescing "injection wheals." If the lesions are so small or so few that 5 cubic centimeters cannot be injected into them, the balance is given intramuscularly. The combination of the intramuscular and intradermal administration in each patient seems desirable. The frequency of injections of any one lesion is limited by the local inflammatory reaction.

III. TREATMENT OF LEPRA REACTION

The following methods have been used in controlling lepra reaction.

(*a*) The patient should be put at rest, and if there is no contraindication, a sharp purgative is given, means being taken thereafter to keep the bowels well regulated.

(*b*) A light but well-balanced, nutritious diet should be given.

(*c*) Accompanying diseases should be searched for and treated. The presence of such diseases, though often obscure, may stimulate a lepra reaction.

(*d*) Potassium antimony tartrate given every second day intravenously in doses of 0.02 to 0.04 gram has been found useful.

(*e*) Calcium chloride (20 cubic centimeters of a 5 per cent solution) may be given intravenously.

(*f*) Calcium lactate, 1 to 2 grams daily in divided doses, and sufficient sodium bicarbonate may be given to make and maintain an alkaline reaction in the urine.

(*g*) Lepra reaction may be accompanied by a very painful neuritis, which may lead to rapid atrophic changes in the parts supplied by the affected nerves. Rapid relief of pain may be afforded by the intramuscular injection of adrenaline (0.3 cubic centimeter of a 1,000 solution) diluted in saline, or by the administration orally of 0.05 gram of ephedrine sulphate. The effect of the latter drug is more lasting than that of the former. Instantaneous relief has also been obtained by the injection around a subcutaneous nerve trunk of 10 cubic centimeters of 0.05 gram of ephedrine sulphate dissolved in saline.

In giving special antileprosy treatment care should be taken not to exceed the tolerance of the patient, otherwise lepra reaction may result. Such special treatment should always be discontinued during lepra reaction and until the patient has completely recovered therefrom.

IV. MANUFACTURE OF MIXED ETHYL ESTERS OF HYDNOCARPUS-GROUP OILS

Preparation of esters by hot process.—Ten liters of hydnocarpus oil are boiled vigorously for forty-eight hours with 5 liters of 96 per cent ethyl alcohol and 100 cubic centimeters of concentrated sulphuric acid under a reflux condenser. The principal advantage of vigorous boiling is undoubtedly that it keeps the two layers well mixed. The esters are then separated from the lower layer, from which the excess alcohol can be recovered by distillation. The esters are washed three times with about 20 liters of water, separated from the washing water, and dried in a steam kettle or on a water bath. The yield from 10 liters of oil is about 11 liters of esters. Proportionally smaller amounts of materials may be used to prepare smaller lots of esters.

Distillation.—The washed and dried ethyl esters are then distilled at 20 to 25 millimeters pressure in a glass or iron still.

Washing with alkali.—Before neutralizing the esters, the acidity is determined by titration. Five hundred grams of lye (94 per cent sodium hydrate) are dissolved in 80 liters of hot water (90° C.) in a 160-liter steel drum. Forty-five liters of the distilled ethyl esters (2.5 to 3 per cent acidity) are added and thoroughly mixed. If the acidity is higher than 3 per cent a correspondingly increased amount of lye is added. After the mixture has stood for twenty-four hours the clear lower layer is drawn off through an outlet in the bottom of the tank. Hot water (90° C.) is added to the 140-liter mark, thoroughly mixed, allowed to settle for twenty-four hours, and again drawn off. The washing with water is repeated four times, and each time twenty-four hours are allowed for settling. The yield is about 96 per cent, and the acidity (as oleic acid) is usually under 0.1 per cent. If smaller lots of esters are to be neutralized it may be found necessary to add crystals of sodium chloride to assist the separation. Heating on a boiling-water bath will also hasten the process.

Steaming and drying.—Volatile impurities, which are often irritating, may be eliminated by blowing out with steam. About 15 liters of the washed esters are put in a 26-liter stoneware carboy and steam is passed through the liquid for two hours or longer, until the pungent odor has disappeared from the condensed steam. The esters are placed in ordinary 5-gallon oil tins to settle, poured off from the separated water, and filtered through paper. The product is a clear, very pale yellow, mobile liquid with a slight, characteristic odor. Smaller quantities of esters may be refined by this method.

Ethyl esters manufactured by the above process are of constant composition and relatively nonirritating, regardless of the source or quality of the original oil.

Preparation of esters by cold process.—Four hundred twenty-five grams of cold-drawn hydnocarpus oil of good quality, 550 cubic centimeters of 96 per cent ethyl alcohol, and 32 cubic centimeters of sulphuric acid (sp. gr. 1.845) are placed in a bottle with a tight-fitting glass stopper and left until the process of esterification is complete. The bottle should be shaken once or twice a day to mix the upper and lower layers. This, or placing the bottle in the sun or in some warm place, hastens the process. Neither shaking nor heat is, however, essential if time is not a consideration. At first the oil forms a lower and the alcohol and acid an upper layer. As esterification proceeds a point is reached at which the lower layer, now chiefly composed of esters, is of lower specific gravity than, and rises above, the original upper layer, which now contains a large proportion of glycerol. To ensure the completion of esterification it is well to allow the process to continue for a further period equal to the time required for the rising of the lower layer. Thus, if the lower layer takes fourteen days to rise, the ingredients should be left in the bottle for another fourteen days. When the upper layer dissolves completely in alcohol the esterification is complete; esters, but not oil, being completely soluble in alcohol. The lower layer is then drawn off and the upper layer repeatedly washed with an equal volume of water until the water is free from acid as tested by litmus paper. A 0.1 per cent solution of sodium hydrate in water is then added; this forms a thick emulsion. The vessel is slowly rotated and crystals of common salt are gradually added in small quantities in order to break the emulsion. The lower layer is removed; and the upper layer consisting of esters, after being washed once more with distilled water, is filtered through thick filter paper. The esters, though now clear, still contain a certain amount of fine emulsion which makes them dark in color. This may be removed by drying on a water bath for two or three hours while stirring constantly with a glass rod. The esters are then filtered again and the process is complete.

The esters may be washed in the bottle in which they have been prepared by substituting for the glass stopper a perforated cork with two glass tubes; one, 2 inches in length, is inserted flush with the inner end of the cork and fitted with a piece of rubber tubing compressed with a spring clamp; the other reaches to the bottom of the bottle. By inverting the bottle the esters rise to the top and the lower layer may be drained off by opening the clamp; or the lower layer may be syphoned off through the long glass tube without inverting the bottle. A separating funnel is more convenient for separating and washing the esters.

Preparation of ethyl esters by hot process without distillation.—The esters may be much more rapidly prepared by placing the ingredients mentioned under the cold process in a flask on a water bath arranged to maintain a constant level of water. A reflux condenser is connected with the flask. The water bath is kept at a temperature sufficient to maintain brisk boiling inside the flask. This is continued without stopping for eighteen hours, when it will be found that esterification is complete. Washing of the esters is then carried out as described in the cold process. The weight of the esters recovered is almost equal to that of the oil used.

In order to produce nonirritating ethyl esters by the last two processes, the original oil must be nonirritating.

Addition of iodine.—Addition of metallic iodine (0.5 per cent) to the ethyl esters markedly reduces the irritating quality of the ethyl esters. Fifteen liters of the purified esters are heated in a 20-liter enameled kettle to 140° C. The esters must be thoroughly dried before iodine is added since, if water is present, it effects by catalysis the hydrolysis of several per cent of the esters. If the filtered esters are clear, the heating to 140° C. before adding the iodine will drive off all dissolved water. Seventy-five grams of chemically pure resublimed iodine are added with stirring. The temperature immediately rises to 150° C., at which point it is maintained for exactly thirty minutes, the liquid being stirred occasionally. After cooling, the iodized esters are filtered into bottles (250 cubic centimeters capacity) and sterilized for one hour in an oven at 150° C. The temperature of the contents of the bottles reaches in this time 110° C. The bottles are tightly corked, and sealed with paraffin or sealing wax and allowed to stand two weeks before use.

Note 1.—Smaller lots of esters can be iodized by this method provided that a shallow (pan) type of container is used.

Note 2.—It is not advisable to use esters after the lapse of two years, although samples that have stood longer than three years do not seem to have deteriorated sufficiently to become markedly irritating.

Note 3.—Iodized esters must be kept tightly corked and preferably in a dark, cool place. Repeated resterilization should be avoided. Heat or sunlight in the presence of air produces a change in the iodized esters, which increases their irritating qualities to a marked extent.

Refining of hydnocarpus-group oils.—Crude hydnocarpus oils are sometimes found to give pain upon injection. A bland, nonirritating oil may be produced by the following method of refining.

Volatile impurities can be removed by passing steam through the oil for about an hour, either before or after washing with alkali. Sufficient steam is used to give an aqueous distillate of about one-fifth the volume of the oil. The amount of volatile impurities is very small, but the distillate has a strong odor.

The free fatty acids are removed by washing with a solution of caustic alkali, and the only difficulty is the separation of the pure oil from the resulting emulsion. The following method has proven satisfactory. One hundred fifty liters of hot water (about 90° C.) are run into a 400-liter steel drum provided with a faucet at the bottom; 0.5 kilogram of lye (94 per cent sodium hydrate) is added, followed by 100 liters of hydnocarpus oil (not over 2.5 per cent acidity), and thoroughly mixed. With oil of higer acidity a correspondingly increased amount of lye must be used.

After the emulsion has stood twenty-four hours the clear lower layer (about 125 liters) is drawn off. Hot water is run in, while stirring, up to the 350-liter mark.

After two days the slightly opalescent lower layer (200 liters) is drawn off. The washing with water is repeated four times, and twenty-four hours are allowed for separation on each occasion.

Ninety-five liters of oil are obtained, with an acidity of not over 0.2 per cent. This oil is steamed as described above and filtered, while hot, through folded filter papers. The filtrate is dried by heating in an enamel-ware kettle, filtered again, and sterilized in bottles at 150° C.

Some provision must be made for keeping the oil hot during the separation of the emulsion if smaller quantities are refined, otherwise the mixture cools too rapidly to allow separation to take place.

V. EPIDEMIOLOGICAL SURVEYS

The data that should be included in an epidemiological survey are given below in tabular form.

OUTLINE OF DATA TO BE OBTAINED

1. Community. Name and type (village, town, district, country, etc.).
    A. Geography. Location; topography; geology (soils, etc.).
    B. Climatology. Temperature (maximum, minimum, mean); humidity (relative, absolute); rainfall (minimum, maximum, mean, seasonal); winds (velocity, prevailing, hurricanes, typhoons, etc.).

- C. Population. Total number and variation in numbers; censuses or estimates; migrations; number of families.
    - 1. Racial. Numbers of each; types of each (aborigines, natives, recent immigrants); mixtures (numbers and types).
    - 2. Social. Religions, castes, etc.; numbers of each, types of each; marriage laws, food laws, etc.; housing: types and sanitary surroundings.
- D. Occupation. Industrial; agriculture (crops, amounts and types); tenant system (serfdom, overcrowding, communal system, family or larger units); husbandry; fisheries.
- E. Diseases (general, not leprosy). Prevailing types, incidence of these and frequency of epidemics; endemicity; diseases probably due to faulty diets, type of diseases and prevalence.
- F. Leprosy. Incidence; distribution (geographically and by house within the community; note any unusual prevalence along lines of communication or in other districts).
- G. School survey. Children; number of children from 1 to 4 years of age (inclusive); number of children of school age; number attending school; number of cases of leprosy in these groups.

2. Family.
    - A. Blood relations. Name of head of family; number in the family; age of members, sex of members; race; number, age and sex of breadwinners; age of individuals and total for family.
    - B. Household family. Same data as for A.
    - C. Diseases. History of diseases other than leprosy in the family, including those prevailing in previous generation. Prevalence of disease in the habitation. Sanitary surroundings of houses.
3. Individual leprosy case.
    - Name; age; sex; occupation (indicate the type of the labor performed); age; economic status; marital state; race; caste; class; religion; education.
    - Dietary; foods and proportions of these used; manner of preparation (freshness of food).
    - Diseases other than leprosy; history, prevalence, etc.; estimate of state of general health.
    - Leprosy; examine for the following; type (neural or cutaneous and degree of these); probable infectivity; source of infection; history of case; history of previous cases in the family, in relatives and previous generations; contacts, including previous cases in the family or household, as well as casual contacts.

THE INTERNATIONAL LEPROSY ASSOCIATION

It was realized that this Conference afforded an exceptional opportunity to consider the question of a permanent international organization of those engaged in activities concerning leprosy and of others interested in such work. It has long been felt that such a body would serve a useful purpose in stimulating greater interest in the problem and in efforts to obtain more effective results.

Accordingly, information was laid before the Conference concerning two movements to this end. These were (1) a discussion begun in 1922–23 among certain leprologists, which resulted in a decision to organize a society as soon as there should seem to be a possibility of obtaining funds to support a periodical; and (2) a separate movement started in 1925–26 to form an organization to be called "Société Internationale de Leprologie." The latter has unfortunately not become active. The Conference was also advised that the Leonard Wood Memorial for the Eradication of Leprosy would consider sympathetically a request for a subsidy to aid such a plan as the Conference might indorse.

After a thorough discussion of the matter it was unanimously decided that the existing circumstances made it advisable to inaugurate a movement in which due consideration should be given the plans of those previously interested in the matter and every effort made to secure their coöperation. A proposed constitution and by-laws, comprising features of the earlier plans, were studied by the Conference. Upon reaching agreement on these, the members of the Conference joined unanimously in forming the Association. A meeting of the Association was then held; the constitution and by-laws, subject to modification when organization of the Association has been completed, were adopted, and temporary officers were elected.

The name of the organization is THE INTERNATIONAL LEPROSY ASSOCIATION (Association Internationale de la Lèpre).

The purposes are: "To encourage and facilitate mutual acquaintance and collaboration between persons of all nationalities concerned in leprosy work and the coördination of their efforts; to facilitate the dissemination of knowledge of leprosy and its control; and in any other practicable manner to aid in the anti-leprosy campaign throughout the world; and to this end to publish a scientific journal of leprosy. It shall endeavor to coöperate with any other institution or organization dealing with leprosy work."

For purposes of expediency provision is made for a Western Section and an Eastern Section; a General Council to be in charge of the general affairs of the association, and Section Councils to be in charge of sectional affairs.

Membership may be of two classes. "Regular membership" is open to all persons with recognized medical or other scientific qualifications, who are actively engaged in connection with the study, treatment, or control of leprosy, or who have been so engaged. "Associate membership" is open to all other persons connected with or especially interested in leprosy.

The chief activities anticipated are those of the holding of meetings and the publishing of a scientific periodical on leprosy. When found desirable and practicable, it is intended that general leprosy congresses shall be convened. It is believed that the sections can hold meetings more frequently, possibly in connection with other medical gatherings.

The proposed periodical, to be called THE INTERNATIONAL JOURNAL OF LEPROSY, will contain, besides suitable original articles, reprintings of important papers that have appeared elsewhere, abstracts, news items, and possibly correspondence and other appropriate material. The editorial control of the Journal will be carried out by a board consisting of an Editor and two Associate Editors, to be chosen by the General Council.

It was agreed that, for some time, it would probably be impossible to finance a satisfactory journal solely from membership fees and subscriptions. Since there was reason to believe that the Leonard Wood Memorial would consider sympathetically a request for a subsidy for this purpose, a resolution was passed requesting such aid for a period of five years. If this is granted, it is intended that publication will commence early in 1932.

The temporary general council elected is: President, Dr. Victor G. Heiser; Vice President, Western Section, Prof. Carlos Chagas; Vice President, Eastern Section, Dr. E. Muir; Secretary, Dr. R. G. Cochrane; Treasurer, Dr. Wm. H. Brown; General Councillors, Prof. Nagayo, Sir Leonard Rogers, Dr. Etienne Burnet, and Prof. de Langen. The council made the following appointments: Editor (and general councillor *ex officio*), Dr. H. W. Wade; Associate Editors, Dr. H. P. Lie and Dr. James L. Maxwell. Elections and appointments of persons not at the meeting are subject to their acceptance.

The temporary council was charged with the duty of enrolling additional members. Upon the attainment of an adequate membership, it will conduct an election of the regular officers, through the post; and shall organize the Sections. It is believed that this can be accomplished within a year. Applications for copies of the constitution and by-laws or other information should be addressed to the Secretary, International Leprosy Association, 29 Dorset Square, London, NW. 1, England.

RESOLUTIONS

RESOLUTION I

Requesting from the Leonard Wood Memorial for the Eradication of Leprosy aid to the International Leprosy Association for the publication of a Journal of Leprosy.

(Adopted, session of January 21, 1931.)

"WHEREAS, the Leonard Wood Memorial Conference on Leprosy, believing it to be of the greatest importance that leprologists and others interested in leprosy be organized and that a scientific journal of leprosy be published, has organized an International Leprosy Association, one of the primary purposes of which shall be to publish an International Journal of Leprosy, and,

"WHEREAS, it is estimated that such a Journal, of satisfactory quality, can be published at a cost of from $5,000 to $6,000 gold a year, but it is recognized that sufficient funds cannot be raised for the purpose previous to publication and that it is uncertain whether for the first few years income (membership fees, subscriptions, advertising) will equal the cost of publication; *Be it*

"RESOLVED, That the Leonard Wood Memorial for the Eradication of Leprosy be approached to grant to the International Leprosy Association a subsidy for five years, the amount to be, for the first year $5,000 gold and for the next four years the amount required to cover the difference between income and cost of publication but not to exceed $5,000 in any one year."

RESOLUTION II

Bringing the suggestion to the notice of The Leonard Wood Memorial for favorable consideration the publication and distribution of a booklet containing plans, photographs, and suitable text of the leprosarium and skin dispensary at Cebu, Philippine Islands.

(Adopted, session of January 21, 1931.)

"This Conference, being convinced of the value of early publication and general distribution to leprosy workers of a booklet containing plans, photographs, and suitable text of the new leprosarium and skin dispensary at Cebu, wishes to take this opportunity of bringing this suggestion to the notice of the Leonard

Wood Memorial for favorable consideration as an activity in the leprosy field which it might find it possible to undertake at an early date."

RESOLUTION III

Indorsing the note on prophylaxis prepared by the Leprosy Commission of the League of Nations at its meeting at Bangkok in December, 1930.

(Adopted, session of January 21, 1931.)

"This Conference has had the opportunity to examine the note on prophylaxis of the Leprosy Commission of the League of Nations prepared at its meeting at Bangkok in December, 1930.

"After consideration thereof, this Conference endorses this report in principle."

RESOLUTION IV

Conveying appreciation of members of Conference to the Board of Directors of the Leonard Wood Memorial for the Eradication of Leprosy, etc.

(Adopted, session of January 21, 1931.)

"This Conference wishes to place on record its high sense of appreciation of the generosity of the Board of Directors of the Leonard Wood Memorial for the Eradication of Leprosy in enabling such a conference to be held in such a highly organized center of antileprosy work as the Philippine Islands.

"The Conference would further desire to record its appreciation of the foresight and action of the Board of Directors of the Leonard Wood Memorial in assembling a representative body of those interested in the treatment of this disease.

"The Conference feels that the results of its deliberations are likely to provide necessary stimulus to the movement for the control and eradication of leprosy with which the Leonard Wood Memorial is so closely identified.

"The Conference wishes further to express its gratitude to the Leonard Wood Memorial for its sympathetic attitude to certain proposals which may require further financial consideration."

The Conference also passed a resolution expressing its grief at the early death of Dr. Eloy V. Pineda, of the leprosy staff of the Philippine Health Service; and a further resolution of appreciation to Mr. Perry Burgess for organizing the Conference and the part he played in its functioning.

ERRATA

Page 253–270, disregard all figure references to plates except those included under "Illustrations" on page 271.

Page 253, in line 16, *for* (1925) *read* (1915).

Page 273, in lines 26 and 27, *for Euparyphium ilocanum= Echinostoma ilocanum* (Garrison, 1908), *read Euparyphium ilocanum* [=*Echinostoma ilocanum* (Garrison, 1908)].

Page 282, in lines 13 and 14, *for Euparyphium ilocanum, Echinostoma ilocanum* (Garrison, 1908), *read Euparyphium ilocanum* [=*Echinostoma ilocanum* (Garrison, 1908)].

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[New names and new combinations are printed in **boldface.**]

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